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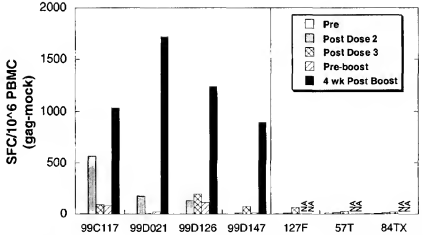
(54) Title: METHOD OF INDUCING AN ENHANCED IMMUNE RESPONSE AGAINST HIV



(57) Abstract: An efficient means of inducing an immune response against human immunodeficiency virus ("HIV") utilizing specific prime-boost regimes is disclosed. The specific prime-boost regimes employ a heterologous prime-boost protocol wherein recombinant adenoviral and poxvirus vectors comprising exogenous genetic material encoding a common HIV antigen are administered in that order. Vaccines administered into living vertebrate tissue in accordance with the disclosed regimes, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance. express the HIV-1 antigen (*e.g.*, Gag), inducing a cellular immune response which specifically recognizes HIV-1. It is believed that the disclosed prime/boost regime will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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TITLE OF THE INVENTION

METHOD OF INDUCING AN ENHANCED IMMUNE RESPONSE AGAINST HIV

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional applications U.S. Serial Nos. 60/363,870 and 60/392,581, filed March 13, 2002 and June 27, 2002, respectively, hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to an enhanced means for inducing an immune response against human immunodeficiency virus ("HIV") utilizing recombinant adenoviral and poxvirus vectors comprising exogenous genetic material encoding an HIV antigen in a heterologous prime-boost administration in the order specified. Applicants have found that the poxvirus administration in this scheme very effectively boosts the adenovirus-primed immune response against HIV. Viruses of use in the instant invention can be any adenovirus or poxvirus, provided that the specific virus utilized is capable of effecting expression of exogenous genetic material introduced into the viral sequence. It is, further, imperative that the virus be replication-defective, host restricted, or modified such that the virus does not freely replicate within the cells of a treated mammalian host. Specific embodiments of the instant invention employ an adenovirus vehicle which is replication-defective and specifically devoid of E1 activity in the priming administration. Further specific embodiments of the instant invention employ modified vaccinia viruses (such as Modified Vaccinia Virus Ankara ("MVA"), or NYVAC, a highly attenuated strain of vaccinia virus) in the boosting administration. Alternative embodiments employ, for instance, a poxvirus selected from the group consisting of canarypoxviruses (such as ALVAC), other fowlpoxviruses and cowpoxviruses. Applicants have found that administration of a recombinant adenoviral vehicle comprising exogenous genetic

material encoding an antigen (specifically, an HIV antigen) followed by subsequent administration of recombinant poxvirus comprising the antigen notably amplifies the response from the initial administration(s) over and above that observed when the antigen is delivered via the recombinant adenoviral or poxviruses independently for both priming and boosting administrations, hence, offering an enhanced immune response. The effective boosting of the adenovirus-primed immune response with poxvirus leads to a significantly enhanced immune response capable of specifically recognizing HIV which is particularly manifest in the cellular immune response. Based on the above findings, it is believed that the disclosed prime/boost regime will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

Effective treatment regimes for HIV-1 infected individuals have become available. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. For instance, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the

kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. $CD8^+$ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive $CD8^+$ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal induction of CTL responses usually requires "help" in the form of cytokines from $CD4^+$ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

Adenoviral vectors have been developed as live viral vectors for delivery and expression of various foreign antigens including HIV and have proven to be effective in eliciting a CTL response in treated individuals. Adenoviruses are non-enveloped viruses containing a linear double-stranded genome of about 36 kb. The vectors achieve high viral titres, have a broad cell tropism, and can infect nondividing cells. Adenoviral vectors are very efficient gene transfer vehicles and are frequently used in clinical gene therapy studies. In addition, adenovirus has formed the basis of many promising viral immunization protocols.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including

env or *gag*. Various treatment regimes based on these vectors were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions, for instance, in the E1 region constitute a safer alternative to their replicating counterparts. Recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see, e.g.,* Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Vaccinia virus and other poxviruses (*e.g.,* avipoxviruses) have been disclosed as promising vaccine candidates for their demonstrated high-level expression of proteins and have been considered recently for the delivery and expression of HIV antigens. Poxviruses are large, enveloped viruses with double-stranded DNA that is covalently closed at the ends. These viruses possess a high insertion capacity for multiple foreign genes and obtain high level cytoplasmic expression of exogenous foreign genetic material. Their use as vaccines has been known since the early 1980's; *see, e.g.,* Panicali *et al.*, 1983 *Proc. Natl. Acad. Sci. USA* 80:5364-5368. Live recombinant vaccines have been tested in clinical trials using recombinant vaccinia virus or canarypoxvirus for expression of the HIV-1 envelope, and the major Epstein-Barr virus membrane glycoprotein or the rabies virus glycoprotein for the induction of immune responses; *e.g.,* Paoletti, 1996 *Proc. Natl. Acad. Sci. USA* 93:11349-53; Gu *et al.*, 1995 *Dev. Biol. Stand.* 84:171-7; and Fries *et al.,* 1996 *Vaccine* 14:428-34.

Administration protocols employing viral vaccine vectors to date have employed various prime-boost inoculation schemes. Two general schemes frequently used are: (1) wherein both priming and boosting of the mammalian host is accomplished using the same virus vehicle, and (2) wherein the priming and boosting is carried out utilizing different vehicles not necessarily limited to virus vehicles. Examples of the latter are, for instance, a scheme composed of a DNA prime and viral boost, and one composed of a viral prime and a viral boost wherein alternate virus are used. Recently, a prime-boost regime of the latter scheme employing a combination of two of the above viruses, adenovirus and poxvirus, in varying order (*i.e.,*

adenovirus-prime, poxvirus-boost; and poxvirus-prime, adenovirus-boost) was utilized to effect the delivery and expression of the CS gene of *Plasmodium berghei* (Ad-PbCS) to mice; Gilbert *et al.*, 2002 *Vaccine* 20:1039-45. This strategy was disclosed to be protective in mice against malaria; *see, e.g.*, Gilbert *et al.*, 2002 *Vaccine* 20:1039-45.

It would be of great import in the battle against AIDS to develop a prophylactic- and/or therapeutic-based HIV vaccine strategy capable of generating a strong cellular immune response against HIV infection. The present invention addresses and meets these needs by disclosing a heterologous prime-boost HIV immunization regime based on the administration of recombinant adenoviral and poxvirus vectors comprising exogenous genetic material encoding a common HIV antigen. The specific prime-boost vaccination regime is one wherein an individual is primed with the recombinant adenoviral vector and then provided a boosting dose of the recombinant poxvirus vector. A vaccine protocol in accords with this description, as far as Applicants are aware, has not been demonstrated for HIV. This vaccine prime-boost regime may be administered to a host, such as a human.

SUMMARY OF THE INVENTION

The present invention relates to an enhanced method for generating an immune response against human immunodeficiency virus ("HIV"). The method is based on the heterologous prime-boost administration of recombinant adenoviral and poxvirus vectors comprising heterologous genetic material encoding an HIV antigen to effect a more pronounced immune response against HIV than that which can be obtained by either vector independently in a single modality prime-boost immunization scheme. A mammalian host is first administered a priming dose of adenovirus comprising a gene encoding the HIV antigen and, following some period of time, administered a boosting dose of poxvirus carrying the gene encoding the HIV antigen. There may be a predetermined minimum amount of time separating the administrations, which time essentially allows for an immunological rest. In particular embodiments, this rest is for a period of at least 4 months. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. Applicants have found that boosting of the adenovirus-primed response with poxvirus in this manner leads to a notably

amplified immune response to the HIV antigen. Thus the instant invention relates to the administration of adenovirus and poxvirus HIV vaccines in this manner.

Accordingly, the instant invention relates to a method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host comprising the steps of (a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter (b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof.

The adenoviral and poxvirus vectors utilized in the immunization regimes of the present invention may comprise any replication-defective adenoviral vector and any replication-defective, replication-impaired or host-restricted poxvirus vector which is genetically stable through large scale production and purification of the virus. In other words, recombinant adenoviral and poxvirus vectors suitable for use in the methods of the instant invention can be any purified recombinant replication-defective, replication-impaired or host-restricted virus shown to be genetically stable through multiple passages in cell culture which remains so during large scale production and purification procedures. Such a recombinant virus vector and harvested virus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of an immunization regime which is based on the use of recombinant replication-defective adenovirus and poxvirus vectors of decreased virulence.

Poxviruses have been the subject of various genetic engineering efforts designed to reduce the virulence of the virus. For instance, efforts with vaccinia virus targeted the viral thymidine kinase, growth factor, hemagglutinin, 13.8 kD secreted protein and ribonucleotide reductase genes; *see* Buller *et al.*, 1985 *Nature* 317(6040):813-815; Buller *et al.*, 1988 *J. Virol.* 62(3):866-74; Flexner *et al.*, 1987 *Nature* 330(6145):259-62; Shida *et al.*, 1988 *J. Virol.* 62(12):4474-80; Kotwal *et al.*, 1989 *Virology.* 171(2):579-87; and Child *et al.*, 1990 *Virology* 174(2):625-9. Modified vaccinia viruses form the subject of, *inter alia*, U.S. Patent Nos. 5,185,146; 5,110,587; 4,722,848; 4,769,330; 5,110,587; and 4,603,112. Avipoxviruses also are

of interest as they possess a limited host range and, therefore, do not freely replicate in human cells. Recombinant avipoxviruses are the subject of, *inter alia*, U.S. Patent Nos. 5,505,941; 5,174,993; 5,942,235; 5,863,542; and 5,174,993. U.S. Patent No. 5,266,313 discloses a raccoon poxvirus-based vaccine for rabies virus. The poxvirus vector of choice is administered to boost the immune response activated by the prior administration of an adenovirus vehicle carrying an HIV transgene.

Adenoviral vectors of use in the instant invention are those that are at least partially deleted in E1 and devoid of E1 activity. Vectors in accordance with this description can be readily propagated in E1-complementing cell lines, such as PER.C6® cells.

The recombinant adenoviral and poxvirus vectors of use in the instant application comprise a gene encoding an HIV antigen. In specific embodiments, the gene encoding the HIV antigen or immunologically relevant modification thereof comprises codons optimized for expression in a mammalian host (*e.g.*, a human). In preferred embodiments, the adenoviral and/or poxvirus vectors comprise a gene expression cassette comprising (a) a nucleic acid encoding an HIV antigen (*e.g.*, an HIV protein) or biologically active and/or immunologically relevant portion/modification thereof; (b) a heterologous (non-native) or modified native promoter operatively linked to the nucleic acid of part a); and, (c) a transcription termination sequence; provided that any promoter utilized to drive expression of the nucleic acid included within the gene expression cassette for the recombinant poxvirus vector is either native to, or derived from, the poxvirus of interest or another poxvirus member. Naturally occurring, nonoverlapping, tandem early/late promoters of moderate strength have been described for vaccinia virus (*see, e.g.*, Cochran, *et al.*, 1985 *J. Virol.* 54:30-37; and Rosel *et al.*, 1986 *J. Virol.* 60:436-9) and have been used for gene expression.. An example of a modified native promoter is the synthetic early/later promoter of Example 2, previously described in Chakrabarti *et al.*, 1997 *BioTechniques* 23(6):1094-97. A heterologous promoter can be any promoter under the sun (modified or not) which is not native to, or derived from, the virus in which it will be used. Preferably, the gene expression cassette used within the recombinant poxvirus comprises (a) a nucleic acid encoding an HIV antigen (*e.g.*, an HIV protein) or biologically active and/or immunologically relevant portion/modification thereof; and (b) a heterologous promoter (from another poxvirus species) or a promoter which is native to or derived from the poxvirus of interest.

HIV antigens of use in the instant invention include the various HIV proteins, immunologically relevant modifications, and immunogenic portions thereof. The present invention, thus, encompasses the various forms of codon-optimized HIV-1 gag (including but by no means limited to p55 versions of codon-optimized full length ("FL") Gag and tPA-Gag fusion proteins), HIV-1 pol, HIV-1 nef, HIV env, fusions of the above constructs, and selected modifications of the above possessing immunological relevance. Examples of HIV-1 Gag, Pol, Env, and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH_2-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

Recombinant viral vectors in accordance with the instant disclosure form an aspect of the instant invention. Other aspects of the instant invention are host cells comprising said adenoviral and/or pox virus vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral and/or pox virus vector into a host cell, and (b) harvesting the resultant vectors.

The present invention also relates to prime-boost regimes wherein the recombinant adenoviral and poxvirus vectors comprise various combinations of the above HIV antigens. Such HIV immunization regimes will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include viral vector-based multivalent vaccine compositions which provide for a divalent (*e.g.*, gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (*e.g.*, gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component. To this end, preferred vaccine compositions for use within the instant methods are adenovirus and poxvirus vectors comprising multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regime.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a recombinant viral vector comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, or possibly a "2+1" divalent vaccine comprising, for instance, a gag-pol fusion (*e.g.*,, codon optimized p55 gag and inactivated optimized pol) within the same backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES).

Administration of the recombinant adenoviral and poxvirus vectors via the disclosed heterologous means provides for improved cellular-mediated immune responses; responses that are more pronounced than that afforded by single modality regimes. An effect of the improved vaccine (adenoviral HIV prime and poxvirus HIV boost) should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. The administration, intracellular delivery and expression of the vaccine in this manner elicits a host CTL and Th response. The individual vaccinee or mammalian host (as referred to herein) can be a primate (both human and non-human) as well as any non-human mammal of commercial or domestic veterinary importance.

In light hereof, the present invention relates to methodology regarding administration of the adenoviral and poxvirus vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. Such treatment regimes may include a monovalent or multivalent composition, and/or various combined modality applications. Therefore, the present invention provides for methods of using the disclosed HIV vaccine administration scheme within the various parameters disclosed herein as well as any additional parameters known in the art which, upon introduction

into mammalian tissue, induces intracellular expression of the HIV antigen(s) and an effective immune response to the respective HIV antigen(s).

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given the recombinant adenovirus and poxvirus HIV vaccines in accordance with the disclosed heterologous prime-boost immunization regime.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective. They typically have a deleted or inactivated E1 gene region, and often have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to base pairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication-deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results in a protein having an N-terminal peptide extension, often referred to as a pro-sequence.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and therefore not transcribed into mRNA or translated into protein.

"Immunologically relevant" or "biologically active," when used in the context of a viral protein, means that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual. The same terms, when used in the context of a nucleotide sequence, means that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to a bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the tissue plasminogen activator leader sequence and an optimized HIV gag gene.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector which is deleted of E1, and contains adenoviral base pairs 1-450 and 3511-3523, with a human codon-optimized HIV-1 gag gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-

bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation.

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intron A) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from base pairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA".

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human

codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the HIV-1 gag adenovector "Ad5HIV-1gag". This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999, and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 1) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the transgene construct disclosed in PCT International Application No. PCT/US01/28861, filed September 14, 2001 in comparison with the original gag transgene. PCT International Application No. PCT/US01/28861 claims priority to U.S. Provisional Application Serial Nos. 60/233,180, 60/279,056, and 60/317,814, filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively; the above applications all of which are hereby incorporated by reference.

Figure 4 shows the modifications made to the adenovector backbone of Ad5HIV-1gag in the generation of the vector disclosed in PCT International Application No. PCT/US01/28861 which is utilized in certain examples of the instant application.

Figure 5 shows the levels of Gag-specific T cells in rhesus macaques immunized with (a) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag and a single booster shot with 10e9 vp MRKAd5 HIV-1 gag ("10e9 vp MRKAd5-10e9 vp MRKAd5"); (b) two priming doses of 10e9 pfu MVA HIV-1 gag and a single booster with 10e9 pfu MVA HIV-1 gag ("10e9 pfu MVA-10e9 pfu MVA"); or (c) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag followed by a single booster shot with 10e9 pfu MVA HIV-1 gag ("10e9 vp MRKAd5-10e9 pfu MVA"). The levels expressed as number of spot-forming cells (SFC) per million PBMC are the mock-corrected values for each animal prior to the start of the immunization regimen ("Pre"); 4 weeks after the first priming dose ("Post Dose 1"); 4 weeks after the second

priming dose ("Post Dose 2"); just prior to the boost ("Pre-Boost"); 4 weeks after the boost ("4 wks Post-Boost"); and 8 weeks after the boost ("8 wks Post-Boost"). For #99D241, data at 4 weeks post boost were unavailable (NA) because of poor PBMC yields.

Figure 6 shows the Gag-specific T cell responses induced by two priming doses of 10e7 vp dose of MRKAd5 HIV-1 gag (week 0; week 4) followed by administration of 10e7 vp MVA HIV-1 gag at week 27. The levels provided are the mock-corrected levels for each animal prior to the start of the immunization regimen ("Pre"); 4 weeks after the first priming dose ("Post Dose 1"); 4 weeks after the second priming dose ("Post Dose 2"); just prior to the boost ("Pre-Boost"); 4 weeks after the boost ("4 wk Post-Boost"); and 8 weeks after the boost ("8 wk Post-Boost"). One will note a significant increase compared to the levels just prior to the boost. MVA-HIVgag elicited a large amplification of the priming response, with levels reaching as high as 1000 SFC/10e6 PBMCs. Because the dose of MVA used as a booster shot induced weak or undetectable immune response in naïve animals (see Figure 5), the post-boost increases shown is largely attributed to the expansion of memory T cells instead of priming of new lymphocytes.

Figure 7 shows ELISPOT responses in BALB/c mice immunized with (1) one dose of 5x10e8 vp Ad5 HIV-1 gag ("Ad5 prime-no boost"), (2) one dose of 5x10e8 vp Ad5 HIV-1 gag followed by one dose of 5x10e6 pfu vaccinia-gag ("Ad5 prime-Vacc Boost"), or (3) one dose of 5x10e6 pfu vaccinia-gag ("Vacc prime-no boost"); Ad5-gag being the original gag vector discussed throughout the specification. The response in totally naïve animals was also assayed. Shown are the mock-corrected frequencies of T cells specific for a defined gag CD8+ epitope in BALB/c mice (AMQMLKETI). Ad5-primed immune responses (about 300 per million) were boosted significantly by administration of vaccinia-gag (to about 1400 per million).

Figure 8 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 9A-1 to 9A-45 illustrate the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:2 [coding] and SEQ ID NO:3 [non-coding]).

Figure 10 shows the levels of Gag-specific antibodies in rhesus macaques immunized with (a) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag and a single booster shot with 10e9 vp MRKAd5 HIV-1 gag ("10e9 vp MRKAd5-10e9 vp MRKAd5"), (b) two priming doses of 10e9 pfu MVA HIV-1 gag and a single booster

with 10e9 pfu MVA HIV-1 gag ("10e9 pfu MVA-10e9 pfu MVA"), or (c) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag followed by a single booster shot with 10e9 pfu MVA HIV-1 gag ("10e9 vp MRKAd5-10e9 pfu MVA"). Shown are the geometric mean titers for each cohort at the start of the immunization regimen ("Pre"), 4 weeks after the first priming dose ("Wk 4"), 4 weeks after the second priming dose ("Wk 8"), just prior to the boost ("Pre-Boost"), and 8 weeks after the boost ("Post-Boost").

Figure 11 shows the homologous recombination protocol utilized to recover pAd6E1-E3+ disclosed herein

Figure 12 shows the levels of Gag-specific T cells in rhesus macaques immunized with three doses of either MRKAd5-HIVgag or MRKAd6-HIVgag followed by a single booster shot with 10^8 pfu of ALVAC-HIVgag (see Table 4). Also shown are the responses in macaques given three (3) doses of 10^9 pfu ALVAC-HIVgag. The levels shown are the mock-corrected levels for each animal prior to the start of the immunization regimen ("Pre"), 4-8 wks after the second priming dose ("Post Dose 2"), 8 wks after the third vaccine dose ("Post Dose 3"), just prior to the boost ("Pre-Boost"), and 4 wks after the boost ("4 wk Post Boost"). For the 127F, 57T, and 84TX subjects, no vaccine (NA-not available) was given after the third ALVAC dose.

DETAILED DESCRIPTION OF THE INVENTION

An enhanced means for generating an immune response against human immunodeficiency virus ("HIV") is described. The method is based on a heterologous prime-boost immunization scheme employing recombinant adenovirus and poxvirus vectors comprising exogenous genetic material encoding an HIV antigen (or antigens) of interest. A priming dose of the HIV antigen(s) is first delivered with a recombinant adenoviral vector. This dose effectively primes the immune response so that, upon subsequent identification of the antigen in the circulating immune system, the immune response is capable of immediately recognizing and responding to the antigen within the host. The priming dose(s) is then followed up with a boosting dose of a recombinant poxvirus vector comprising exogenous genetic material encoding the antigen. It has been found that, as relates to HIV antigens, administration in accordance with this description results in a significant non-additive synergistic effect which notably increases the immune response seen in inoculated

mammalian hosts. The effects are particularly evident in the cellular immune responses generated following inoculation. The disclosed immunization regime, thus, offers a prophylactic advantage to previously uninfected individuals and can offer a therapeutic effect to reduce viral load levels in those already infected with the virus, hence prolonging the asymptomatic phase of HIV-1 infection.

Accordingly, the instant invention relates to a method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host comprising the steps of (a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter (b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; said recombinant poxvirus vector being replication-impaired in the mammalian host. "Replication-impaired" in this context has a broad meaning and generally describes (1) those vectors that have been attenuated or modified such that replication is not possible; (2) those vectors that have been attenuated or modified such that replication is impaired; and (3) those vectors that simply do not replicate, or replicate at a much reduced level, in the particular mammalian species that is treated. Replication of avipoxviruses, for instance, appears to be restricted to avian species. For this reason, avipoxviruses stand as a very safe vector for use in mammals. Replication appears to be blocked at a step prior to viral-DNA synthesis, presumably allowing for the use of only the early promoters; *see, e.g.,* Moss, B., 1993 *Curr. Opin. Genet. Devel.* 3:86-90; and Taylor *et al.*, 1991 *Vaccine* 9:190-3. This level of replication has, however, been noted to afford protective immunization; *see, e.g.,* Wild *et al.*, 1990 *Vaccine* 8:441-442; and 1992 *Virology* 187:321-28; and Cadoz *et al.*, 1992 *Lancet* 339:1429-32. Poxviruses form an essential element of the instant methods as they have been found to exhibit a surprising ability to significantly boost an adenoviral-primed immune response against HIV. Specific embodiments of the instant invention employ modified vaccinia viruses (such as Modified Vaccinia Virus Ankara ("MVA"), subject of U.S. Patent No. 5,185,146; and NYVAC, a highly attenuated strain of vaccinia virus disclosed in, *inter alia*, Tartaglia *et al.*, 1992 *Virology* 188:217-232) in the boosting administrations of the instant invention, although any poxvirus and, particularly vaccinia virus, that can effectuate the delivery and expression of an

antigen of interest and which is of reduced virulence in the intended mammalian host is encompassed herein. Modified vaccinia viruses and their use in various methods have been disclosed in the art, *see, e.g.*, U.S. Patent Nos. 5,185,146; 5,110,587; 4,722,848; 4,769,330; 5,110,587; and 4,603,112. This is true as well for generalized methods for constructing recombinant vaccinia virus; *see, e.g.*, Earl *et al.*, In *Current Protocols in Molecular Biology*, Ausubel *et al.*eds., New York: Greene Publishing Associates & Wiley Interscience; 1991:16.16.1-16.16.7. Further embodiments of the instant application utilize alternative poxvirus vectors in the boosting administration of the disclosed methods. Of specific mention, are avipoxviruses such as ALVAC (the subject of, *inter alia*, U.S Patent Nos. 5,505,941; 5,174,993; 5,942,235; 5,863,542; and 5,174,993). ALVAC, as indicated earlier, is a plaque-purified clone derived from an attenuated canarypox virus obtained from the wild-type strain after 200 passages in chick embryo fibroblasts. ALVAC recombinants and the use thereof form another aspect of the instant invention. A specific example of such an ALVAC recombinant is vCP 205. vCP 205 (ATCC Acc. No. VR-2547) is, in brief, an ALVAC recombinant (ALVAC-MN120TMG) which expresses HIV1 (IIIB) gag (and protease) proteins, as well as a form of the HIV1(MN) envelope glycoprotein in which gp120 is fused to the transmembrane anchor sequence derived from gp41. Incorporation of the HIV genes in an ALVAC backbone is described in issued U.S. Patent No. 5,863,542 (*see, e.g.*, Example 14). The recombinant canarypox virus ALVAC-HIV (vCP205) was obtained by homologous recombination between the pHIV32 plasmid and the ALVAC genomic DNA. The pHIV32 plasmid encodes the HIV-1 gp120-MN and the anchoring region of gp41 (transmembrane glycoprotein of HIV-1 gp41 LAI), the Gag p55-polyprotein, and the protease-LAI whose expressions are under control of the HG and I3L vaccinia promoters, respectively. The nucleotide sequence of the H6-promoted HIV1 gp120 (+transmembrane) gene and the I3L-promoted HIV1gag(+pro) gene contained in pHIV32 is disclosed in Figures 14A to 14C of U.S. Patent No. 5,863,542 which is hereby incorporated by reference.. Deletion of the ectodomain of gp41 is believed to make it easier to distinguish between infected and vaccinated subjects since most HIV-infected subjects show antibodies directed against the immunodominant region of gp41 precisely deleted in vCP205.

Strategies involved in the construction of recombinant poxvirus are known, *see, e.g.*, Panicali & Paoletti, 1982 *Proc. Natl. Acad. Sci. USA* 79:4927-31; Nakano *et*

al., 1982 *Proc. Natl. Acad. Sci. USA* 79:1593-96; Piccini *et al.*, In *Methods in Enzymology*, Wu & Grossman, eds., Academic Press, San Diego, 153:545-63; U.S. Patent No. 4,603,112; Sutter *et al.*, 1994 *Vaccine* 12:1032-40; and Wyatt *et al.*, 1996 *Vaccine* 15:1451-8. Methods for creating synthetic recombinant poxviruses are also described in U.S. Patent Nos. 4,769,330; 4,722,848; 4,603,112; 5,110,587; and 5,174,993 ; the disclosures of which are incorporated herein by reference. The construction of recombinant MVA and ALVAC recombinant virus comprising exogenous genetic material coding for HIV gag is described herein in Examples 2 and 10, respectively. As one of ordinary skill in the art will appreciate, insertion of the exogenous genetic material can be targeted to numerous locations of the poxvirus genome provided the location does not negate the ability of the virus to effect expression of the genetic material. In order to ensure the infectivity of the virus and, hence, expression of the construct, insertion must occur into silent regions of the genome or into nonessential genes. The recombinant MVA constructs disclosed herein, for instance, have the exogenous genetic material incorporated into the thymidine kinase region and the deletion II region (a region defined, *inter alia*, in Meyer *et al.*, 1991 *J. Gen. Virol.* 72:1031-8); *see* Example 2.

Recombinant adenoviral vectors form an essential element of the methods of the instant invention as they have been found to very effectively prime the immune response against a specific antigen of interest. Preferred embodiments of the instant invention employ adenoviral vectors which are replication-defective by reason of having a deletion in/activation of the E1 region which renders the vector devoid (or essentially devoid) of E1 activity. Adenovirus serotype 5 has been found to be a very effective adenovirus vehicle for purposes of effectuating sufficient expression of exogenous genetic material (particularly HIV antigens) in order to provide for sufficient priming of the mammalian host immune response. Alternative replication-defective adenoviral vehicles capable of effecting expression of the HIV antigen are, however, also suitable for use herein.

The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is an immunization scheme employing a vector based on the wildtype adenovirus serotype 5 sequence in the priming administration; a virus of which has been deposited with the American Type Culture Collection ("ATCC") under ATCC Deposit No. VR-5.

One of skill in the art can, however, readily identify alternative adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42) and incorporate same into the disclosed heterologous prime-boost immunization schemes. Accordingly, the instant invention encompasses methods employing all adenoviral vectors partially deleted in E1 in the administration schemes of the instant invention.

Recombinant adenoviral vectors comprising deletions additional to that contained within the region of E1 are also contemplated for use within the methods of the instant invention. For example, vectors comprising deletions in both E1 and E3 are contemplated for use within the methods of the instant invention. Such a vector can accommodate a larger amount of foreign DNA inserts (or exogenous genetic material).

Adenoviral vectors of use in the methods of the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

Adenoviral pre-plasmids (*e.g.*, pMRKAd5gag) can be generated by homologous recombination using adenovirus backbones (*e.g.*, MRKHVE3) and the appropriate shuttle vector. The plasmid in linear form is capable of replication after entering the PER.C6® cells, and virus is produced. The infected cells and media are then harvested after viral replication is complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6®. Both these cell lines express the adenoviral E1 gene product. PER.C6® is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6®, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is preferred that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

Adenoviral and poxvirus vectors of use in the instant invention comprise a gene encoding an HIV-1 antigen or an immunologically relevant modification thereof. HIV antigens of interest include, but are not limited to, the major structural proteins of HIV such as Gag, Pol, and Env, immunologically relevant modifications, and immunogenic portions thereof. The invention, thus, encompasses the various forms of codon-optimized HIV-1 gag (including but by no means limited to p55 versions of codon-optimized full length ("FL") Gag and tPA-Gag fusion proteins), HIV-1 pol, HIV-1 nef, HIV env, and selected modifications of immunological relevance. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous (non-native) or modified native promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription termination sequence; provided that any promoter utilized to drive expression of the nucleic acid included within the gene expression cassette for the recombinant poxvirus vector is either native to, or derived from, the poxvirus of interest or another poxvirus member. Naturally occurring, nonoverlapping, tandem early/late promoters of moderate strength have been described for vaccinia virus (*see, e.g.,* Cochran, *et al.*, 1985 *J. Virol.* 54:30-37; and Rosel *et al.*, 1986 *J. Virol.* 60:436-9) and have been used for gene expression. An example of a modified native promoter is the synthetic early/later promoter of Example 2, previously described in Chakrabarti *et al.*, 1997 *BioTechniques* 23(6):1094-97. Preferably, the gene expression cassette used within the recombinant poxvirus comprises (a) a nucleic acid encoding an HIV antigen (*e.g.,* an HIV protein) or biologically active and/or immunologically relevant portion thereof; and (b) a heterologous promoter (from another poxvirus species) or a promoter which is native to or derived from the poxvirus of interest.

The transcriptional promoter of the recombinant adenoviral vector is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV),

constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 μg and 200 μg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter. In preferred embodiments, the promoter may comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought. Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:4). A recombinant adenoviral vectors with an expression cassette comprising a CMV promoter (devoid of the intron A region) and a BGH terminator forms a specific aspect of the present invention, although other promoter/terminator combinations can be used. Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA.

Recombinant viral vectors in accordance with the instant disclosure form an aspect of the instant invention. Other aspects of the instant invention are host cells comprising said adenoviral and/or pox virus vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral and/or pox virus vector into a host cell, and (b) harvesting the resultant vectors.

Administration of the viral vectors in accordance with the methods of the instant invention should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen

(e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be incorporated into the recombinant viral vectors of use in the methods of the instant invention, preferred embodiments include the codon optimized p55 gag antigen, pol and nef. The adenoviral and/or pox virus vehicles of the instant invention can utilize heterologous nucleic acid which may or may not be codon-optimized. In specific embodiments of the instant invention, the individual can be primed with an adenoviral vector comprising codon-optimized heterologous nucleic acid, and boosted with a pox virus vector comprising non-codon-optimized nucleic acid. Administration of multiple antigens possesses the possibility for exploiting various different combinations of codon-optimized and non-codon-optimized sequences.

Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the viral vaccines will encode modified versions of pol or nef. Preferred embodiments of the viral vaccines carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. A clade B or clade C based p55 gag antigen will potentially be useful on a global scale. A transgene of choice for insertion into the vectors utilized within the disclosed methods is a codon-optimized version of p55 gag.

In addition to a single HIV antigen of interest being delivered by the adenoviral and poxvirus vectors, two or more antigens can be delivered either via separate vehicles or delivered *via* the same vehicle. For instance, a priming dose in accordance with the instant invention can comprise a recombinant viral vector comprising genes encoding both nef and pol or, alternatively, two or more alternative HIV-1 antigens. The boosting dose could then comprise a recombinant poxvirus vector comprising the genes encoding both nef and pol (or whichever two or more HIV-1 antigens were used in the priming dose). In an alternative scenario, the priming dose can comprise a mixture of separate adenoviral vehicles each comprising a gene encoding for a different HIV-1 antigen. In such a case, the poxvirus boosting dose would also comprise a mixture of poxvirus vectors each comprising a gene encoding for a separate HIV-1 antigen, provided that the boosting dose administers recombinant viral vectors comprising genetic material encoding for the same antigens that were delivered in the priming dose. Alternatively, a poxvirus vector expressing all HIV-1 antigens could be generated to serve as a boosting agent for vaccination. These divalent (*e.g.*, gag and nef, gag and pol, or pol and nef components) or trivalent (*e.g.*, gag, pol and nef components) vaccines can further be administered by a combination of the techniques described above. Therefore, a preferred aspect of the present invention are the various vaccine formulations that can be administered by the methods of the instant invention. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen.

The disclosed immunization regimes employing fusion constructs composed of two or more antigens are also encompassed herein. For example, multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-viral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, or possibly a "2+1" divalent vaccine comprising, for instance, a gag-pol fusion (*e.g.*,, a codon optimized p55 gag and inactivated optimized pol) with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames in the same construct may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. In the absence of the use of IRES-based technology, it is

preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may include a three transgene vector such as that wherein a gagpol fusion and nef gene were included in the same vector with different promoters and termination sequences being used for the gagpol fusion and nef gene. Further, potential "2+1" divalent vaccines of the present invention might be wherein a single construct containing gag and nef with separate promoters and termination sequences is administered in combination with a construct comprising a pol gene with promoter and termination sequence. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These compositions are, as above, preferably delivered along with a viral composition comprising an additional HIV antigen in order to diversify the immune response generated upon inoculation. Therefore, a multivalent vaccine delivered in a single, or possibly second, viral vector is certainly contemplated as part of the present invention. It is important to note that, in terms of deciding on an insert for the recombinant adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the viral vehicle. Adenovirus, for instance, has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred in certain embodiments that the sequence be "optimized" for expression in a mammalian (*e.g.*, human cellular environment, particularly in the adenoviral constructs. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon

frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeast and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is a vaccine administration protocol wherein the adenoviral and poxvirus vectors both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol, env, or nef, although as stated above, one or more of the viral vehicles of the instant invention can utilize heterologous nucleic acid which may or may not be codon-optimized. In specific embodiments of the instant invention, the individual can be primed with an adenoviral vector comprising codon-optimized heterologous nucleic acid, and boosted with a pox virus vector comprising non-codon-optimized nucleic acid. Administration of multiple antigens possesses the possibility for exploiting various different combinations of codon-optimized and non-codon-optimized sequences.

A vaccine composition comprising the recombinant viral vectors either in the priming or boosting dose in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation for

the recombinant adenoviral vector has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM $MgCl_2$; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used to make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM $MgCl_2$, 0.005% polysorbate 80 at pH 8.0. This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of viral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of $1x10^7$ to $1x10^{12}$ particles and preferably about $1x10^{10}$ to $1x10^{11}$ particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The administration schemes of the instant invention are based on the priming of the immune response with an adenoviral vehicle comprising a gene encoding an HIV antigen (or antigens) and, following a predetermined length of time, boosting the adenovirus-primed response with a poxvirus vector comprising a gene encoding an HIV antigen(s). Multiple primings, typically, 1-4, are usually employed, although more may be used. The length of time between prime and boost may typically vary from about four months to a year, but other time frames may be used. The booster dose may be repeated at selected time intervals.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV but remain uninfected; CTL has been noted in

several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

HIV-1 Gag Gene

A synthetic gene for HIV gag from HIV-1 strain CAM-1 was constructed using codons frequently used in humans; *see* Korber *et al.*, 1998 *Human Retroviruses and AIDS*, Los Alamos Nat'l Lab., Los Alamos, New Mexico; and Lathe, R., 1985 *J. Mol. Biol.* 183:1-12. Figure 2 illustrates the nucleotide sequence of the exemplified optimized codon version of full-length p55 gag. The gag gene of HIV-1 strain CAM-1 was selected as it closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence (Los Alamos HIV database). Advantage of this "codon-optimized" HIV gag gene as a vaccine component has been demonstrated in immunogenicity studies in mice. The "codon-optimized" HIV gag gene was shown to be over 50-fold more potent to induce cellular immunity than the wild type HIV gag gene when delivered as a DNA vaccine.

A KOZAK sequence (GCCACC) was introduced proceeding the initiating ATG of the gag gene for optimal expression. The HIV gag fragment with KOZAK sequence was amplified through PCR from V1Jns-HIV gag vector. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; *see* Montgomery *et al.*, 1993 *DNA Cell Biol.* 12:777-783, for a description of the plasmid backbone.

EXAMPLE 2

Recombinant MVA Construction And Purification

Two recombinant MVA constructs were constructed with the HIV gag gene fragment with KOZAK sequence cloned into two different locations of the MVA genome, the viral thymidine kinase region (MVA-HIV gag TK) and the deletion II region (MVA-HIV gag dII), respectively, with the appropriate linker sequence of the restriction sites. The thymidine kinase region insertion was achieved through the use of shuttle vector pSC59 (*see*, Chakrabarti *et al.*, 1997 *BioTechniques* 23(6):1094-1097) with the HIV gag fragment inserted at a unique *Xho* I site. The deletion II region insertion was accomplished through the use of pLW21 wherein the HIV gag fragment was inserted at a unique *Pme*I site. pLW21 is basically a plasmid derived from pGEM4 vector (Promega) containing a single synthetic early/late promoter and a unique *Pme*I site for cloning. The promoter and cloning site are flanked by MVA viral sequence on both sides for targeted insertion upon homologous recombination events into the deletion II region of the MVA genome Expression of the transgene within both constructs is driven by a synthetic early/late promoter previously described for vaccinia virus (Chakrabarti et al, *supra*). Viral transcription termination and polyadenylation signal sequences were not included in the inserted fragment, as sequences native to the flanking regions of the insert were generally considered sufficient for the transcription termination and polyadenylation of transgene transcript (*see* B Moss, Current Topics in Microbiology and Immunology, 158:25, 1992). The authenticity of the transgene product expressed through the poxvirus vector was guaranteed by the translational termination codon (TAA) at the 3' end of transgene ORF. The orientation and authenticity of the insertions were confirmed by DNA sequencing.

Methods for generating recombinant MVA have been described previously (*see, e.g.*, Sutter *et al.*, 1994 *Vaccine* 12:1032-1040; Wyatt *et al.*, 1996 *Vaccine*, 15:1451-1458). Briefly, sub-confluent primary chick embryo fibroblast cells (CEF) in 25 cm^2 cell culture flask were infected with wild-type MVA at a multiplicity of infection ("m.o.i.") of 0.05 for two hours, and were then transfected with approximately 20 mcg of shuttle vector DNA precipitated with Lipofectin (GIBCO BRL). The cells were cultured for two days, and then the cell pellets were lysed in 1 ml PBS/BSA by repeated freezing-thawing. The cell lysate was used to infect CEFs

in a 6-well plate at dilutions of 1:3, 1:9 and 1:27 in duplicates. After two days, the medium was removed and the cell monolayers were washed twice with PBS. The cells were then frozen and thawed three times and the plaques containing cells infected with recombinant MVA were identified by immunostaining, with sequential incubations with a monoclonal antibody against HIV gag (Advanced Biotechnology Inc) and goat-anti-mouse IgG antibody conjugated with peroxidase (Pierce) with *o*-dianisidine as substrate. The blue plaques formed by the infected cells were picked under the inverted microscope, and the cells were diluted in 1 ml PBS. The cells were lysed by freezing-thawing, and the recombinant MVA was further purified in CEF, using dilutions of 1:5, 1:20 and 1:80, for another 5 rounds. The recombinant MVA was then expanded in CEF in a tissue culture flask of 25 cm^2, and the expression of HIV gag was confirmed by Western blot analysis in CV-1 cells infected with MVA at different dilutions. The final viral stock was prepared in 40 to 80 flasks of 150 cm^2 of CEF, and the viral titers were determined by plaque assay using an immunostaining method.

Recombinant MVA constructs with insertion into the deletion II region were used in the immunizations discussed below.

EXAMPLE 3

Generation of Adenoviral Vector Constructs

A. Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIJnsHIVgag was used as the starting material to amplify the hCMV promoter. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *Msc*1 site of the hCMV promoter and a 3' primer (designed to contain the B*gl*II recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *Msc*1 and B*gl*II. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *Msc*1 and *Bgl*II digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA

expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *Bgl*II digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *Bgl*II site. Colonies were screened using *Sma*1 restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

B. Construction of the Modified Shuttle Vector -"MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from base pairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

(1) The left ITR region was extended to include the *Pac*1 site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.

(2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.

(3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbone pAdHVE3 by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

C. Construction of Modified Adenovector Backbone

An original adenovector pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region) was reconstructed so that it would contain the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with *Pac*1 and *Bst*Z1101 and

isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from *Cla*1 linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from the transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from the transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovector was designated MRKpAdHVE3 (E3+ plasmid). Virus from the new adenovector (MRKHVE3) as well as the old version were generated in the PER.C6® cell lines. In addition, the multiple cloning site of the original shuttle vector contained ClaI , BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVE3 pre-plasmid along with the modification made to the packaging region and pIX gene.

D. Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *Msc*1 overnight and then digested with *Sfi*1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 minutes at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 minutes at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel orientation.

E. Construction of the MRK FG Adenovector

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*1. The reaction mixture was digested with *BsfZ*171. The 5,291 bp fragment was purified

by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH_20. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from the transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size.

F. Virus generation of an enhanced adenoviral construct – "MRK Ad5 HIV-1 gag"

MRK Ad5 HIV-1 gag contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac*1 to release the vector backbone and 3.3 µg was transfected by the calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6® cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [^{33}P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried

down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac*I/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

All viral constructs (adenovirus and poxvirus) were confirmed for Gag expression by Western blot analysis.

EXAMPLE 4

Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

EXAMPLE 5

ELISPOT Assay

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-amino acid ("aa") peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μL of 2-4 x 10^5 peripheral blood mononuclear cells (PBMCs) were added. The cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 μL of media or the gag peptide pool at 8 μg/mL concentration per peptide were added to the PBMC. The samples were incubated at 37°C, 5% CO_2 for 20-24 hrs. Spots were developed accordingly and the plates were processed using

custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD). The counts were normalized to 10^6 cell input.

EXAMPLE 6

Anti-p24 ELISA

A modified competitive anti-p24 assay was developed using reagents from the Coulter p24 Antigen Assay kit (Beckman Coulter, Fullerton, CA). Briefly, to a 250-μL serum sample, 20 μL of Lyse Buffer and 15 μL of p24 antigen (9.375 pg) from the Coulter kit were added. After mixing, 200 μL of each sample were added to wells coated with a mouse anti-p24 mAb from the Coulter kit and incubated for 1.5 hr at 37°C. The wells were then washed and 200 μL of Biotin Reagent (polyclonal anti-p24-biotin) from the Coulter kit was added to each well. After a 1 hr, 37°C incubation, detection was achieved using strepavidin-conjugated horseradish peroxidase and TMB substrate as described in the Coulter Kit. OD_{450nm} values were recorded. A 7-point standard curve was generated using a serial 2-fold dilution of serum from an HIV-seropositive individual. The lower cut-off for the assay is arbitrarily set at 10 milli Merck units/mL (mMU/mL) defined by a dilution of the seropositive human serum. This cutoff falls at approximately 65% of the maximum bound control signal which corresponds to that obtained with the diluent control only and with no positive analyte.

EXAMPLE 7

Intracellular Cytokine Staining

To 1 ml of 2 x 10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μg/mL. For gag-specific stimulation, 10 μL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hours at 37 °C, 5% CO_2, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20

μL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μL anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 μL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μL 1xFACS Perm buffer (Becton Dickinson) for 10 minutes at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 minutes of incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACS Calibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated and a common fluorescence cut-off for cytokine-positive events was used for both $CD4^+$ and $CD8^+$ populations, and for both mock and gag-peptide reaction tubes of a sample.

EXAMPLE 8

Results

A. Immunization Regimen

Cohorts of 3-6 rhesus macaques were immunized following homologous and heterologous prime-boost regimens involving MRKAd5 and MVA vectors expressing the same codon-optimized HIV-1 gag. The immunization schedule is described in Table 1.

Table 1

Group	Prime	Boost (month 6)
1	10e9 vp MRKAd5-HIVgag at week 0, 4	10e9 vp MRKAd5-HIVgag
2	10e9 pfu MVA-HIVgag at week 0, 4	10e9 pfu MVA-HIVgag
3	10e9 vp MRKAd5-HIVgag at week 0, 4	10e9 pfu MVA-HIVgag

B. T Cell Immune Responses

Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figures 5 and 6. They are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool minus the mock control.

Figure 5 shows the T cell responses induced by (a) two priming immunizations with 10e9 vp MRKAd5 HIV-1 gag followed by a 10e9 vp MRKAd5 HIV-1 gag booster ("10e9 vp MRKAd5-10e9 vp MRKAd5"); (b) two priming doses of 10e9 pfu MVA HIV-1 gag and a single booster with 10e9 pfu MVA HIV-1 gag ("10e9 pfu MVA-10e9 pfu MVA"); or (c) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag followed by a single booster shot with 10e9 pfu MVA HIV-1 gag ("10e9 vp MRKAd5-10e9 pfu MVA"). The rest period between last priming and booster doses varied from 20-23 weeks (20 for the MVA-MVA subjects; 22 for subjects 99D262, 99C117, and 99D227 of the MRKAd5-MRKAd5 group; and 23 for the remaining subjects). Administration of the same dose of MRKAd5 HIV-1 gag at approximately month 6 resulted in slight increases compared to the levels just prior to the boost; the post-boost levels were largely comparable to if not weaker than the peak levels before the boost. This is possibly due to the presence of neutralizing immunity generated against the vector by the first two immunizations. The responses after the boost did not surpass 500 gag-specific T cells per 10e6 PBMC, with a mean of 275 SFC/10e6 PBMC for all 6 monkeys. Monkeys given three of 10e9 pfu MVA HIV-1 gag (at 0, 1, 6 months) exhibited very weak HIV-specific T cells responses not exceeding 100 SFC/10e6 PBMC. In contrast, when both modalities are combined in which animals were given two priming doses of 10e9 vp MRKAd5 HIV-1 gag and a single booster shot of 10e9 pfu MVA HIV-1 gag, the levels of gag-specific T cells increased to peak responses above 1200 SFC/10e6 PBMC for all 3 monkeys. The property of MVA HIV-1 gag to boost effectively MRKAd5-gag-primed immune responses is very striking considering that MVA HIV-1 gag is a rather poor immunogen; it also offers a great advantage compared to boosting with the same MRKAd5 HIV-1 gag. The ability of poxvirus vector to boost primed responses was also evident using a lower priming dose of 10^7 vp of MRKAd5 HIV-1 gag (Figure 6).

PBMCs from the vaccinees of the heterologous MRKAd5 prime-MVA boost regimen were analyzed for intracellular IFN-γ staining after the priming immunizations (week 13) and after the booster immunizations (wk 31). The assay provided information on the relative amounts of $CD4^+$ and $CD8^+$ gag-specific T cells in the peripheral blood (Table 2). The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4+ and CD8+ T cells.

Table 2

			Post Prime		Post Boost	
Prime	Boost	ID	%CD4+	%CD8+	%CD4+	%CD8+
MRKAd5-HIVgag	MVA-HIVgag	99D241	0.00*	0.13	0.08**	0.37**
10^9 vp	10^9 pfu	99D244	0.02	0.09	0.25	0.92
wk 0, 4	wk 27	99D252	0.04	0.08	0.43	0.13

Numbers reflect the percentages of circulating CD3+ lymphocytes that are either gag-specific CD4+ or gag-specific CD8+ cells. Mocks values have been subtracted.
*No detectable antigen-specific CD4+ T cells above background
**Collected at wk 35 instead of wk 31

C. Humoral Immune Responses

The p24-specific antibody titers were determined for each animal at several time points. The geometric mean titers for each cohort were calculated and shown in Figure 10. Two doses of MRKAd5 HIV-1 gag were able to induce moderate levels of anti-p24 antibodies (about 1000 mMU/mL) whereas two doses of MVA did not appear to induce any detectable level of anti-p24 antibodies. Administration of MVA HIV-1 gag boosted the humoral immune responses primed by MRKAd5 HIV-1 gag by about 6-fold (to about 7000 mMU/mL). This booster effect is similar to that elicited by a 10^9 vp dose of MRKAd5 HIV-1 gag. However, the booster effect seen in these animals with 10^9 vp MRKAd5 HIV-1 gag is expected to be lower if the subjects have higher levels of Ad5-directed neutralizing activity due to anamnestic responses to the first two MRKAd5 doses. The booster effect of MVA HIV-1 gag, on the other hand, would not be affected by any pre-existing neutralizing titers directed at Ad5.

EXAMPLE 9

Immunization Regime Using Replication-Proficient Vaccinia Virus

BALB/c mice were vaccinated intramuscularly with one of the following immunization regimes: (1) one priming dose of 5x10e8 vp Ad5-gag (the adenoviral vector disclosed in PCT International Application No. PCT/US00/18332 which is hereby incorporated by reference); (2) one priming dose of 5x10e8 vp Ad5-gag followed by one boosting dose of 5x10e6 pfu vaccinia-gag; or (3) one priming dose of 5x10e6 pfu vaccinia-gag. The response in totally naïve animals was also assayed. Figure 7 shows the mock-corrected frequencies of T cells specific for a defined gag CD8+ epitope in BALB/c mice (AMQMLKETI). The results indicate that the Ad5-

primed immune responses (about 300 per million) were boosted significantly by administration of vaccinia-gag (to about 1400 per million).

While this virus is replication-proficent and hence not suitable for use in the methods of the instant invention (absent modification), Applicants believe that the example serves to demonstrate with a different poxvirus strain how poxvirus very effectively boosts an adenovirus-primed response.

The mice in this example, one will note, were only primed once. Those of skill in the art will appreciate that due consideration must be given to the general observation that these smaller animal systems require less number of immunizations and/or smaller doses to prime the immune compared to larger non-human primates.

EXAMPLE 10

Recombinant ALVAC Construction And Purification

Recombinant ALVAC constructs expressing the codon-optimized human HIV-1 gag open reading frame (SEQ ID NO: 1) were generated in accordance with basic procedure well understood and appreciated in the art; *see, e.g.,* U.S. Patent Nos. 5,863,542 and 5,766,598. The procedure generally entails the placement of a gene sequence of interest (herein, SEQ ID NO: 1) ligated or operatively linked to a promoter of interest (e.g., H6 vaccinia virus early promoter) into a plasmid construct containing DNA homologous to a section of DNA within the poxvirus where insertion is desired. As previously mentioned, this site should not contain an essential locus. Following this first step(s), the resulting plasmid construct is amplified by growth within E. coli bacteria and isolated. The isolated plasmid containing the insert of interest is then transfected into a cell culture, *e.g.,* chick embryo fibroblasts, along with the pox virus of interest (herein, ALVAC). The recombinant viruses are then selected and purified by serial rounds of plaque purification.

EXAMPLE 11

Generation of Adenoviral Serotype 6 Vector Constructs

A. Construction of Ad6 Pre-Adenovirus Plasmid

An Ad6 based pre-adenovirus plasmid which could be used to generate first generation Ad6 vectors was constructed taking advantage of the extensive sequence

homology (approx. 98%) between Ad5 and Ad6. Homologous recombination was used to clone wtAd6 sequences into a bacterial plasmid.

The general strategy used to recover pAd6E1-E3+ as a bacterial plasmid is illustrated in Figure 11. Cotransformation of BJ 5183 bacteria with purified wt Ad6 viral DNA (ATCC Accession No. VR-6) and a second DNA fragment termed the Ad5 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 33798 to 35935) and left (bp 1 to 341 and bp 3525 to 5767) end of the Ad5 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad5 342 to 3524. The Ad5 sequences in the ITR cassette provide regions of homology with the purified Ad6 viral DNA in which recombination can occur.

Potential clones were screened by restriction analysis and one clone was selected as pAd6E1-E3+. This clone was then sequenced in it entirety. pAd6E1-E3+ contains Ad5 sequences from bp 1 to 341 and from bp 3525 to 5548, Ad6 bp 5542 to 33784, and Ad5 bp 33967 to 35935 (bp numbers refer to the wt sequence for both Ad5 and Ad6). pAd6E1-E3+ contains the coding sequences for all Ad6 virion structural proteins which constitute its serotype specificity.

B. Construction of an Ad6 Pre-Adenovirus Plasmid containing the HIV-1 gag gene

(1) *Construction of Adenoviral Shuttle Vector:*

The shuttle plasmid MRKpdelE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA was constructed by inserting a synthetic full-length codon-optimized HIV-1 *gag* gene into MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.). MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) contains Ad5 sequences from bp 1 to 5792 with a deletion of E1 sequences from bp 451 to 3510. The HCMV promoter and BGH pA were inserted into the E1 deletion in an E1 parallel orientation with a unique BglII site separating them. The synthetic full-length codon-optimized HIV-1 *gag* gene was obtained from plasmid pV1Jns-HIV-FLgag-opt by *BglII* digestion, gel purified and ligated into the *BglII* restriction endonuclease site in MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.), generating plasmid MRKpdelE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA. The genetic structure of MRKpdelE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA was verified by PCR, restriction enzyme and DNA sequence analyses.

(2) *Construction of pre-adenovirus plasmid:*

Shuttle plasmid MRKpdelE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA was digested with restriction enzymes *Pac*1 and *Bst*1107I and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I-digested) adenoviral backbone plasmid, pAd6E1-E3+. The genetic structure of the resulting pMRKAd6gag was verified by restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for large-scale production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the gag transgene in transient transfection cell culture.

pMRKAd6gag contains Ad5 bp 1 to 450 and from bp 3511 to 5548, Ad6 bp 5542 to 33784, and Ad5 bp 33967 to 35935 (bp numbers refer to the wt sequence for both Ad5 and Ad6). In the plasmid the viral ITRs are joined by plasmid sequences that contain the bacterial origin of replication and an ampicillin resistance gene.

C. Generation of research-grade recombinant MRKAd6gag

To prepare virus for pre-clinical immunogenicity studies, the pre-adenovirus plasmid pMRKAd6gag was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 10 μg of pMRKAd6gag was digested with restriction enzyme *Pac*I (New England Biolabs) and transfected into a 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested after complete viral cytopathic effect (CPE) was observed. The virus stock was amplified by multiple passages in PER.C6® cells. At the final passage virus was purified from the cell pellet by CsCl ultracentrifugation. The identity and purity of the purified virus was confirmed by restriction endonuclease analysis of purified viral DNA and by gag ELISA of culture supernatants from virus infected mammalian cells grown in vitro. For restriction analysis, digested viral DNA was end-labeled with P^{33}-dATP, size-fractionated by agarose gel electrophoresis, and visualized by autoradiography.

All viral constructs were confirmed for Gag expression by Western blot analysis.

EXAMPLE 12

Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points (typically, four week intervals) during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

EXAMPLE 13

ELISPOT Assay

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749; Casimiro *et al.*, 2002 *J. Virol.* 76:185-94), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-amino acid ("aa") peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 µL of 2-4 x 10^5 peripheral blood mononuclear cells (PBMCs) were added. The cells were counted using a Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 µL of media or the gag peptide pool at 8 µg/mL concentration per peptide were added to the PBMC. The samples were incubated at 37°C, 5% CO_2 for 20-24 hrs. Spots were developed accordingly and counted under microscope. The counts were normalized to 10^6 cell input.

EXAMPLE 14

Intracellular Cytokine Staining

To 1 ml of 2 x 10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293,

Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μg/mL. For gag-specific stimulation, 10 μL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hour, after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hours at 37 °C, 5% CO_2, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 minutes at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 minutes, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μL anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 μL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μL 1xFACS Perm buffer (Becton Dickinson) for 10 minutes at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 minutes of incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACS Calibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated and a common fluorescence cut-off for cytokine-positive events was used for both $CD4^+$ and $CD8^+$ populations, and for both mock and gag-peptide reaction tubes of a sample.

EXAMPLE 15

Results

A. Immunization Regimen

A cohort of four (4) macaques were given three (3) doses of either MRKAd5-HIVgag or MRKAd6-HIVgag at weeks 0, 4, 26. At week fifty-six (56), a booster shot of 10^8 pfu of ALVAC-HIVgag was delivered intramuscularly. For comparison, a separate cohort of three (3) monkeys were given three (3) doses of the same ALVAC-HIVgag (10^9 pfu) at weeks 0, 4, 27. All viral vectors expressed the same codon-optimized HIV-1 gag. The immunization schedule is described in Table 3.

Table 3

Grp	Monkey ID	Vaccine 1	Vaccine 2
1	99C117	10^9 vp MRKAd5-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56
	99D021	10^7 vp MRKAd5-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56
	99D126	10^9 vp MRKAd6-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56
	99D147	10^7 vp MRKAd6-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56
2	127F,57T, 84TX	10^9 pfu ALVAC-HIVgag at wk 0, 4, 27	none

B. T Cell Immune Responses

Vaccine-induced T cell responses against HIV-1 gag were quantified using an IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 12. They are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool minus the mock control.

Figure 12 shows that 10^7-10^9 vp dose of MRKAd5-HIVgag or MRKAd6-HIVgag induced levels of gag-specific T cell responses not exceeding 600 SFC/10^ PBMC. Three out of the four animals had levels below 300 SFC/10^6 PBMC after two doses of the adenoviral-based vaccine. At the time of the ALVAC booster immunization which is about half a year since the last adenovirus dose, antigen-specific responses remained detectable ranging from 10-114 SFC/10^6 PBMC in these animals. However, administration of the ALVAC resulted in about 10-80-fold enhancement in T cell responses when compared to the levels at the time of the booster. These results are very surprising given that ALVAC is intrinsically a rather weak vaccine vector for inducing primary T cell immune response in macaques. Three monkeys that were given multiple immunizations of ALVAC-HIVgag at an even higher dose level (10^9 pfu) exhibited very weak responses to the antigen (less than 100 SFC/10^6 PBMC) (Figure 12).

It is not believed that a fourth immunization with the same adenovirus at an equivalent dose level such as that provided the first three (3) times would be capable of eliciting these large responses because of the potentially significant pre-existing anti-adenovirus immunity generated by the first three (3) doses. Also note that the third adenovirus dose in these monkeys yielded levels that do not even compare to the levels seen following the ALVAC booster. These results clearly show that while ALVAC-based vectors are weak inducers of primary immune response they serve as excellent boosters of existing immune response to an HIV antigen. It also illustrates that a synergy exists between MRKAd-based vectors and ALVAC.

PBMCs from the vaccinees of the heterologous MRKAd5/MRKAd6-ALVAC boost regimens were analyzed for intracellular IFN-γ staining after the boosting immunization (week 60). The assay results provide information on the relative amounts of $CD4^+$ and $CD8^+$ gag-specific T cells in the peripheral blood (Table 4). The results indicate that the heterologous prime-boost immunization approach was able to elicit both HIV-specific CD4+ and CD8+ T cells in rhesus macaques.

Table 4

			Gag-Specific (Wk 60)	
Monkey ID	Vaccine 1	Vaccine 2	%CD4	%CD8
99C117	10^9 vp MRKAd5-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56	0.12	0.26
99D021	10^7 vp MRKAd5-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56	0.08	0.70
99D126	10^9 vp MRKAd6-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56	0.06	0.35
99D147	10^7 vp MRKAd6-HIVgag at wk 0, 4, 26	10^8 pfu ALVAC-HIVgag at wk 56	0.07	0.23

Numbers reflect the percentages of circulating CD3+ lymphocytes that are either gag-specific CD4+ or gag-specific CD8+ cells. Mocks values (less than 0.02%) have been subtracted.

EXAMPLE 16

Immunization and Results

A. Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. ELISPOT Assay

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen et al., 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μL of 2-4 x 10^5 peripheral

blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 fL. Either 50 µL of media or the gag peptide pool at 8 µg/mL concentration per peptide were added to the PBMC. The samples were incubated at 37°C, 5% CO_2 for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10^6 cell input.

C. Intracellular Cytokine Staining

To 1 ml of 2 x 10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 µg/mL. For gag-specific stimulation, 10 µL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 µL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO_2, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 µL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 µL anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 µL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 µL 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 µg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both $CD4^+$ and $CD8^+$ populations, and for both mock and gag-peptide reaction tubes of a sample.

D. Results

Cohorts of 4 monkeys were given at wk 0 one of the following booster vaccines: (A) ALVAC vcp205, 10^8 pfu; (B) ALVAC vcp205, 10^7 pfu; (C) ALVAC HIV-1 gag, 10^8 pfu; (D) ALVAC HIV-1 gag, 10^7 pfu, or (E) MRKAd5

HIV-1 gag, 10^9 vp. ALVAC vcp205 encodes the gene for HIV-1 IIIB gag. ALVAC HIV-1 gag encodes the codon-optimized HIV-1 CAM-1 gag. The animals prior to this immunization had received 3 previous doses of at least 10^9 vp Ad5 HIV-1 gag. The last immunization with Ad5 HIV-1 gag was given more than a year prior. The neutralization titers to Ad5 vector were measured in all animals just prior to wk 0 time point. Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Table 6; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool minus the mock control.

Table 5

					IFN-γ ELISPOT, SFC/10^6 PBMC					
Grp	Booster, Wk 0	Monk ID#	Diff. Days[a]	Ad5 neut[b]	Peak, Prime[c]		T=0 Wk		T=2 Wk	
					Mock	Gag	Mock	Gag	Mock	Gag
1	ALVAC	99C069	617	1065	0	116	0	40	1	584
	vcp205	98X012	848	457	1	121	3	8	3	843
	10^8 pfu	CB4B	695	285	10	330	3	59	15	865
		98X011	695	192	1	361	10	43	3	1205
		Mean[d]	*714*	*404*		*200*		*25*		*841*
2	ALVAC HIV-1 gag	99D193	617	291	4	146	0	34	10	1648
	10^8 pfu	CD1V	617	222	16	251	0	18	13	826
		CB58	617	171	0	265	1	18	5	734
		97N144	848	947	5	373	3	159	0	1838
		Mean[d]	*675*	*320*		*239*		*35*		*1156*
3	MRKAd5-gag	101H	695	490	0	115	3	58	1	696
	10^9 vp	99C213	617	98	11	226	3	14	0	420
		99D137	617	754	8	268	4	49	0	1220
		105F	695	907	5	380	15	76	13	163
		Mean[d]	*656*	*368*		*222*		*36*		*480*

[a]Difference in days between the day of ALVAC boost and the third Ad5 vaccination
[b]Neutralization titers 1 month prior to boost; reported are geometric means of up to 3 measurements
[c]Peak anti-gag T cell responses (SFC/10^6 PBMC) during Ad5 priming vaccinations
[d]Arithmetic means for difference in days; geometric means for Ad5 neut titers; mock-corrected gag T cell responses.

Table 5 shows the T cell responses induced using a homologous boost with MRKAd5-gag or with ALVAC vector. On the basis of the ELISPOT results, it appears that the boosting with ALVAC, specifically ALVAC HIV-1 gag, provides greater booster responses than the MRKAd5-gag.

PBMCs from the vaccinees were analyzed for intracellular IFN-γ staining 2 wks after the booster immunization. This assay provided information on the amounts of $CD4^+$ and $CD8^+$ gag-specific T cells in the peripheral blood (Table 6).

The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4+ and CD8+ T cells. It also indicates that the ALVAC booster induces as much gag-specific CD8+ T cells as MRKAd5gag. However, the ALVAC booster induces higher levels of helper responses than MRKAd5-gag. On the basis of total antigen-specific CD3+ T cells induced as measured by this assay, the ALVAC HIV-1 gag booster shows a statistically significant 6-fold improvement (P=0.004) than the MRKAd5-gag booster.

Table 6

Group	Vaccine	Monk #	CD3+CD4+IFNγ+ per 10^6 Lymp[a]		CD3+CD8+IFNγ+ per 10^6 Lymp[b]		%CD3+CD8+[c]	Total CD3+ 10^6 Lymp[d]
			Mock	Gag	Mock	Gag		
1	ALVAC gag	99C069	129	945	64	462	33.8	1234
	vqq205	98X012	17	1150	60	368	21.7	1460
	10^8 pfu	CB4B	82	1607	100	1203	43.6	2528
		98X011	37	1833	74	656	24.5	2377
		Mean[e]		***1243***		***540***		***1783***
2	ALVAC	99D193	87	6744	104	9479	58.5	16332
	HIV-1 gag	CD1V	0	1877	72	702	25.1	2507
	10^8 pfu	CB56	16	1123	63	2148	65.3	3192
		97N144	80	2231	77	5323	70.7	7417
		Mean[e]		***2341***		***2835***		***5178***
3	MRKAd5	101H	62	266	71	643	73.5	778
	HIV-1 gag	99C213	19	245	48	538	88.4	718
	10^9 vp	99D137	25	158	58	3592	98.4	3888
		105F	34	218	17	218	52.2	384
		Mean[e]		***184***		***668***		***852***

[a]Number of IFN-γ producing CD3+CD4+ cells per million lymphocytes

[b]Number of IFN- γ producing CD3+CD8+ cells per million lymphocytes

[c]Percentage of Gag-Specific T cells that are CD3+CD8+

[d]Sum of IFN- γ producing CD3+CD4+ plus CD3+CD8+ cells per million lymphocytes

[e]Geometric means of mock-corrected values

EXAMPLE 17

Immunization Regimen

Cohorts of 3-6 rhesus macaques will be immunized in accordance with the following homologous and heterologous prime-boost immunization schedule (Table 7), involving Ad5-gag, -pol, and -nef vectors expressing codon-optimized HIV-1 gag, pol and nef, respectively, and ALVAC-gag, pol, nef expressing all three genes in one virus under separate promoter controls. The total dose of each vaccine will be suspended in approximately 1 mL of buffer. The macaques will be anesthetized (ketamine/xylazine) and the vaccines will be delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson,

Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) will be prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment will be in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

Table 7.

Group	Prime	Boost
1	10^9 vp/vector Ad5-gag, -pol, -nef at week 0,4	10^8 pfu ALVAC-gag,pol,nef
2	10^7 vp/vector Ad5-gag, -pol, -nef at week 0,4	10^8 pfu ALVAC-gag,pol,nef
3	10^8 pfu ALVAC-gag,pol,nef at week 0,4	10^7 vp/vector Ad5-gag, -pol, -nef
4	10^9 vp/vector Ad5-gag, -pol, -nef at week 0,4	10^9 vp/vector Ad5-gag, -pol, -nef
5	10^7 vp/vector Ad5-gag, -pol, -nef at week 0,4	10^7 vp/vector Ad5-gag, -pol, -nef
6	10^8 pfu ALVAC-gag,pol,nef at week 0,4	10^8 pfu ALVAC-gag,pol,nef

EXAMPLE 18

SIV Challenge Experiment

Cohorts of 3-6 monkeys will be immunized in accordance with the following heterologous prime-boost immunization schedule (Table 8), involving Ad5-SIV-gag, -pol, and -nef vectors expressing codon-optimized SIV gag, pol and nef, respectively, and ALVAC-SIV gag, pol, nef expressing all three genes in one virus under separate promoter controls. The animals will be pre-screened and distributed for the presence of mamuA01 allele. The total dose of each vaccine will be suspended in approximately 1 mL of buffer. The macaques will be anesthetized (ketamine/xyluzine) and the vaccines will be delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) will be prepared from blood samples collected at several time points during the immunization regimen to monitor for SIV-specific T cell responses. After the ALVAC booster, animals will

be given systemic inoculation of SIVmac239 strain. Animals will be monitored for both virological (i.e., viral loads) and immune parameters (e.g., virus-specific T cell responses, CD4 counts, and lymphoid structures). All animal care and treatment will be in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

Table 8.

Monkey	Prime	Boost	Challen
MamuA01+	10^11 vp/vector Ad5-SIVgag, -SIVpol, -SIVnef at week 0,4	10^8 pfu ALVAC-SIVgag,pol,nef at week 24	SIVmac at week
MamuA01+	None	None	SIVmac at week
MamuA01-	10^11 vp/vector Ad5-SIVgag, -SIVpol, -SIVnef at week 0,4	10^8 pfu ALVAC-SIVgag,pol,nef at week 24	SIVmac at week
MamuA01-	None	None	SIVmac at week

WHAT IS CLAIMED IS:

1. A method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding the HIV-1 antigen or immunologically relevant modification thereof; provided said poxvirus vector is replication-impaired in the mammalian host.

2. A method in accordance with claim 1 wherein the adenoviral vector is of serotype 5.

3. A method in accordance with claim 2 wherein the recombinant adenoviral vector is deleted of base pairs corresponding to base pairs 451-3510 of a wildtype adenovirus serotype 5 genome.

4. A method in accordance with claim 1 wherein the adenoviral vector is of serotype 6.

5. A method in accordance with claim 1 wherein at least one of the genes encoding the HIV-1 antigen or immunologically relevant modification thereof comprises codons optimized for expression in a human.

6. A method in accordance with claim 1 wherein the recombinant adenoviral vector comprises a gene expression cassette comprising:

(a) a nucleic acid encoding an HIV-1 antigen;

(b) a heterologous promoter operatively linked to the nucleic acid encoding the antigen; and

(c) a transcription termination sequence.

7. A method in accordance with claim 1 wherein the recombinant poxvirus vector comprises a gene expression cassette comprising:

(a) a nucleic acid encoding an HIV-1 antigen; and

(b) a promoter operatively linked to the nucleic acid encoding the antigen; provided that said promoter is derived from or native to a poxvirus.

8. A method in accordance with claim 6 wherein the gene expression cassette in the recombinant adenoviral vector is inserted into the E1 region.

9. A method in accordance with claim 8 wherein the gene expression cassette in the recombinant adenoviral vector is in an E1 parallel orientation.

10. A method in accordance with claim 6 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

11. A method in accordance with claim 10 wherein the promoter is an immediate early human cytomegalovirus promoter.

12. A method in accordance with claim 7 wherein the promoter is a synthetic early/late promoter of vaccinia virus.

13. A method in accordance with claim 6 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

14. A method in accordance with claim 6 wherein the HIV-1 antigen is HIV-1 gag.

15. A method in accordance with claim 7 wherein the HIV-1 antigen is HIV-1 gag.

16. A method in accordance with claim 6 wherein the gene expression cassette comprises an open reading frame encoding an HIV-1 gag protein or immunologically relevant modification thereof.

17. A method in accordance with claim 7 wherein the gene expression cassette comprises an open reading frame encoding an HIV-1 gag protein or immunologically relevant modification thereof.

18. A method in accordance with claim 1 wherein the poxvirus vector is attenuated.

19. A method in accordance with claim 1 wherein the poxvirus vector is a vaccinia virus vector modified so as to render the virus replication-defective within the treated mammalian host.

20. A method in accordance with claim 1 wherein the poxvirus vector is an avipoxvirus.

21. A method in accordance with claim 1 wherein the poxvirus vector is a fowlpoxvirus.

22. A method in accordance with claim 1 wherein the poxvirus vector is MVA.

23. A method in accordance with claim 1 wherein the poxvirus vector is the NYVAC strain of vaccinia virus.

24. A method in accordance with claim 1 wherein the poxvirus vector is ALVAC.

25. A method for inducing an enhanced immunological response against an HIV-1 gag antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector of serotype 5 at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 gag antigen or immunologically relevant modification thereof; and thereafter

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof; provided said poxvirus vector is replication-impaired in the mammalian host.

26. A method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant ALVAC vector comprising a gene encoding the HIV-1 antigen or immunologically relevant modification thereof.

27. A method for inducing an enhanced immunological response against an HIV-1 gag antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 gag antigen or immunologically relevant modification thereof; and thereafter

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant ALVAC vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof.

28. A method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter

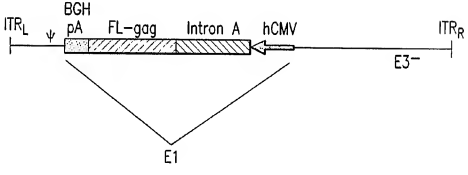
(b) inoculating the mammalian host with a boosting immunization comprising a recombinant MVA vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof.

29. A method for inducing an enhanced immunological response against an HIV-1 gag antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 gag antigen or immunologically relevant modification thereof; and thereafter

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant MVA vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof.

ORIGINAL ADENOVECTOR CONSTRUCT:



ORIGINAL HIV-1 gag ADENOVECTOR.

FIG.1

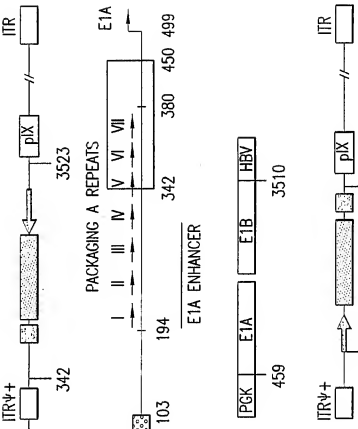
Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg
caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc
ctgctggagacctctgaggggtgcaggcagatcctgggccagctccagccctccctgcaaacaggctctgagg
agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag
gaggccctggagaagattgaggaggagcagaacaagtccaagaaggaaggcccagcaggctgctgctggc
acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag
gccatctccccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctccctgaggtgatccc
catgttctctgcctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagtgggggggccatc
aggctgccatgcagatgctgaaggagaccatcaatgaggaggctgctgagtgggacaggctgcatcctgtgc
acgctggccccattgccccggccagatgagggagcccagggctctgacattgctggcaccacctccaccct
ccaggagcagattggctggatgaccaacaacccccccatccctgtgggggaaatctacaagaggtggatcat
cctgggcctgaacaagattgtgaggatgtactcccccacctccatcctggacatcaggcagggccccaaggag
cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact
ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg
ctgccaccctggaggagatgatgacagcctgccagggggtgggggccctggtcacaaggccagggtgctg
gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag
gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga
agggctgctggaagtgtggcaaggagggccaccagatgaaggactgcaatgagaggcaggccaacttcctg
ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagccccct
cccgaggagtccttcaggtttgggaggagaagaccacccccagccagaagcaggagccccattgacaagg
agctgtacccccctggcctccctgaggtccctgtttggcaacgaccccctcctcccagtaaaataaagccccgggca
gat

FIG.2

DIAGRAMMATIC REPRESENTATION OF THE ORIGINAL HIV-1 GAG TRANSGENE AND THE SERIES OF NEW TRANSGENE CONSTRUCTIONS.

FIG.3



MODIFICATIONS MADE TO THE CURRENT ADENOVECTOR BACKBONE IN THE GENERATION OF THE NEW VECTOR.

FIG.4

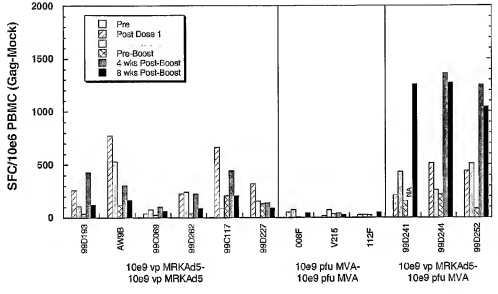

SFC/10e6 PBMC (Gag-Mock)
2000
1500
1000
500
0
Pre
Post Dose 1
Pre-Boost
4 wks Post-Boost
8 wks Post-Boost
980193
AN90
990099
980592
99C117
980227
008F
V216
112F
980241
NA
980244
980252
10e9 vp MRKAd5-
10e9 vp MRKAd5
10e9 pfu MVA-
10e9 pfu MVA
10e9 vp MRKAd5-
10e9 pfu MVA

FIG. 5

Ad5-pox Application

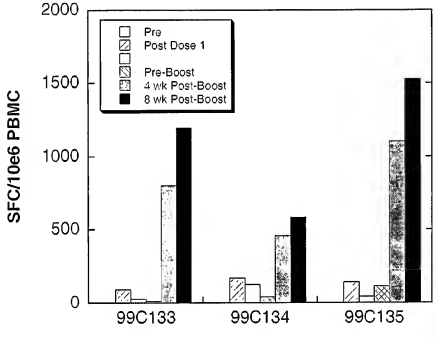


FIG. 6

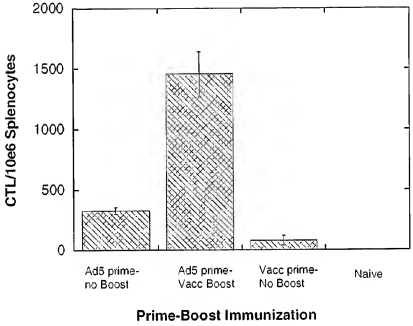

2000
1500
1000
500
0
CTL/10e6 Splenocytes
Ad5 prime-
no Boost
Ad5 prime-
Vacc Boost
Vacc prime-
No Boost
Naive
Prime-Boost Immunization

FIG. 7

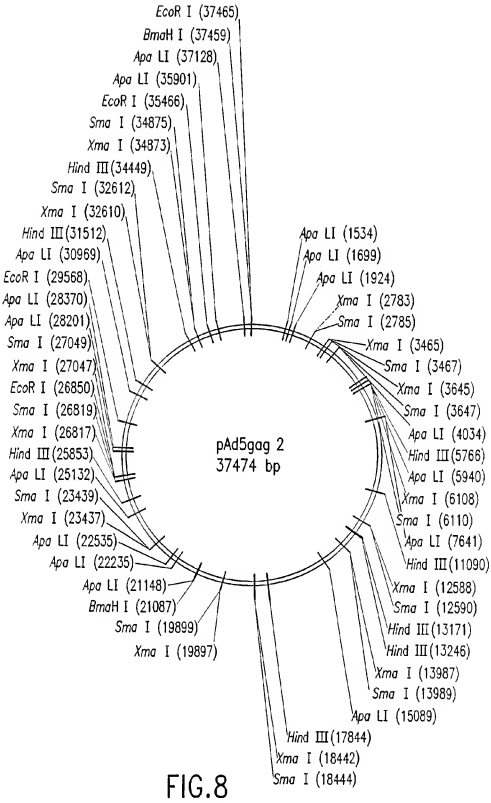

pAd5gag 2
37474 bp
EcoR I (37465)
BmaH I (37459)
Apa LI (37128)
Apa LI (35901)
EcoR I (35466)
Sma I (34875)
Xma I (34873)
Hind III (34449)
Sma I (32612)
Xma I (32610)
Hind III (31512)
Apa LI (30969)
EcoR I (29568)
Apa LI (28370)
Apa LI (28201)
Sma I (27049)
Xma I (27047)
EcoR I (26850)
Sma I (26819)
Xma I (26817)
Hind III (25853)
Apa LI (25132)
Sma I (23439)
Xma I (23437)
Apa LI (22535)
Apa LI (22235)
Apa LI (21148)
BmaH I (21087)
Sma I (19899)
Xma I (19897)
Hind III (17844)
Xma I (18442)
Sma I (18444)
Apa LI (15089)
Xma I (13987)
Sma I (13989)
Hind III (13171)
Hind III (13246)
Xma I (12588)
Sma I (12590)
Hind III (11090)
Apa LI (7641)
Xma I (6110)
Sma I (6108)
Apa LI (5940)
Hind III (5766)
Apa LI (4034)
Xma I (3645)
Sma I (3647)
Xma I (3465)
Sma I (3467)
Apa LI (1534)
Apa LI (1699)
Apa LI (1924)
Xma I (2783)
Sma I (2785)

FIG.8

PacI
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```
1   TTCTTAATTA ACATCATCAA TAATATACCT TATTTTGGAT TGAAGCCAAT
    AAGAATTAAT TGTAGTAGTT ATTATATGGA ATAAAACCTA ACTTCGGTTA

51  ATGATAATGA GGGGGTGGAG TTTGTGACGT GGCGCGGGGC GTGGGAACGG
    TACTATTACT CCCCCACCTC AAACACTGCA CCGCGCCCCG CACCCTTGCC

101 GGCGGGTGAC GTAGTAGTGT GGCGGAAGTG TGATGTTGCA AGTGTGGCGG
    CCGCCCACTG CATCATCACA CCGCCTTCAC ACTACAACGT TCACACCGCC

151 AACACATGTA AGCGACGGAT GTGGCAAAAG TGACGTTTTT GGTGTGCGCC
    TTGTGTACAT TCGCTGCCTA CACCGTTTTC ACTGCAAAAA CCACACGCGG

201 GGTGTACACA GGAAGTGACA ATTTTCGCGC GGTTTTAGGC GGATGTTGTA
    CCACATGTGT CCTTCACTGT TAAAAGCGCG CCAAAATCCG CCTACAACAT

251 GTAAATTTGG GCGTAACCGA GTAAGATTTG GCCATTTTCG CGGGAAAACT
    CATTTAAACC CGCATTGGCT CATTCTAAAC CGGTAAAAGC GCCCTTTTGA

301 GAATAAGAGG AAGTGAAATC TGAATAATTT TGTGTTACTC ATAGCGCGTA
    CTTATTCTCC TTCACTTTAG ACTTATTAAA ACACAATGAG TATCGCGCAT

351 ATATTTGTCT AGGGCCGCGG GGACTTTGAC CGTTTACGTG GAGACTCGCC
    TATAAACAGA TCCCGGCGCC CCTGAAACTG GCAAATGCAC CTCTGAGCGG

401 CAGGTGTTTT TCTCAGGTGT TTTCCGCGTT CCGGGTCAAA GTTGGCGTTT
    GTCCACAAAA AGAGTCCACA AAAGGCGCAA GGCCCAGTTT CAACCGCAAA

451 TATTATTATA GGCGGCCGCG ATCCATTGCA TACGTTGTAT CCATATCATA
    ATAATAATAT CCGCCGGCGC TAGGTAACGT ATGCAACATA GGTATAGTAT

501 ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT
    TATACATGTA AATATAACCG AGTACAGGTT GTAATGGCGG TACAACTGTA

551 TGATTATTGA CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA
    ACTAATAACT GATCAATAAT TATCATTAGT TAATGCCCCA GTAATCAAGT

601 TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC
    ATCGGGTATA TACCTCAAGG CGCAATGTAT TGAATGCCAT TTACCGGGCG

651 CTGGCTGACC GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT
    GACCGACTGG CGGGTTGCTG GGGGCGGGTA ACTGCAGTTA TTACTGCATA

701 GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA
    CAAGGGTATC ATTGCGGTTA TCCCTGAAAG GTAACTGCAG TTACCCACCT

751 GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC
    CATAAATGCC ATTTGACGGG TGAACCGTCA TGTAGTTCAC ATAGTATACG
```

FIG.9A-1
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```
801  CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT
     GTTCATGCGG GGGATAACTG CAGTTACTGC CATTTACCGG GCGGACCGTA

851  TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA
     ATACGGGTCA TGTACTGGAA TACCCTGAAA GGATGAACCG TCATGTAGAT

901  CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA
     GCATAATCAG TAGCGATAAT GGTACCACTA CGCCAAAACC GTCATGTAGT

951  ATGGGCGTGG ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC
     TACCCGCACC TATCGCCAAA CTGAGTGCCC CTAAAGGTTC AGAGGTGGGG

1001 ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC GGGACTTTCC
     TAACTGCAGT TACCCTCAAA CAAAACCGTG GTTTTAGTTG CCCTGAAAGG

1051 AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG
     TTTTACAGCA TTGTTGAGGC GGGGTAACTG CGTTTACCCG CCATCCGCAC

1101 TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC
     ATGCCACCCT CCAGATATAT TCGTCTCGAG CAAATCACTT GGCAGTCTAG

1151 GCCTGGAGAC GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA
     CGGACCTCTG CGGTAGGTGC GACAAAACTG GAGGTATCTT CTGTGGCCCT

1201 CCGATCCAGC CTCCGCGGCC GGGAACGGTG CATTGGAACG CGGATTCCCC
     GGCTAGGTCG GAGGCGCCGG CCCTTGCCAC GTAACCTTGC GCCTAAGGGG

1251 GTGCCAAGAG TGAGATCTAC CATGGGTGCT AGGGCTTCTG TGCTGTCTGG
     CACGGTTCTC ACTCTAGATG GTACCCACGA TCCCGAAGAC ACGACAGACC

1301 TGGTGAGCTG GACAAGTGGG AGAAGATCAG GCTGAGGCCT GGTGGCAAGA
     ACCACTCGAC CTGTTCACCC TCTTCTAGTC CGACTCCGGA CCACCGTTCT

1351 AGAAGTACAA GCTAAAGCAC ATTGTGTGGG CCTCCAGGGA GCTGGAGAGG
     TCTTCATGTT CGATTTCGTG TAACACACCC GGAGGTCCCT CGACCTCTCC

1401 TTTGCTGTGA ACCCTGGCCT GCTGGAGACC TCTGAGGGGT GCAGGCAGAT
     AAACGACACT TGGGACCGGA CGACCTCTGG AGACTCCCCA CGTCCGTCTA

1451 CCTGGGCCAG CTCCAGCCCT CCCTGCAAAC AGGCTCTGAG GAGCTGAGGT
     GGACCCGGTC GAGGTCGGGA GGGACGTTTG TCCGAGACTC CTCGACTCCA

1501 CCCTGTACAA CACAGTGGCT ACCCTGTACT GTGTGCACCA GAAGATTGAT
     GGGACATGTT GTGTCACCGA TGGGACATGA CACACGTGGT CTTCTAACTA

1551 GTGAAGGACA CCAAGGAGGC CCTGGAGAAG ATTGAGGAGG AGCAGAACAA
     CACTTCCTGT GGTTCCTCCG GGACCTCTTC TAACTCCTCC TCGTCTTGTT

1601 GTCCAAGAAG AAGGCCCAGC AGGCTGCTGC TGGCACAGGC AACTCCAGCC
     CAGGTTCTTC TTCCGGGTCG TCCGACGACG ACCGTGTCCG TTGAGGTCGG
```

FIG.9A-2

```
1651  AGGTGTCCCA GAACTACCCC ATTGTGCAGA ACCTCCAGGG CCAGATGGTG
      TCCACAGGGT CTTGATGGGG TAACACGTCT TGGAGGTCCC GGTCTACCAC

1701  CACCAGGCCA TCTCCCCCCG GACCCTGAAT GCCTGGGTGA AGGTGGTGGA
      GTGGTCCGGT AGAGGGGGGC CTGGGACTTA CGGACCCACT TCCACCACCT

1751  GGAGAAGGCC TTCTCCCCTG AGGTGATCCC CATGTTCTCT GCCCTGTCTG
      CCTCTTCCGG AAGAGGGGAC TCCACTAGGG GTACAAGAGA CGGGACAGAC

1801  AGGGTGCCAC CCCCCAGGAC CTGAACACCA TGCTGAACAC AGTGGGGGGC
      TCCCACGGTG GGGGGTCCTG GACTTGTGGT ACGACTTGTG TCACCCCCCG

1851  CATCAGGCTG CCATGCAGAT GCTGAAGGAG ACCATCAATG AGGAGGCTGC
      GTAGTCCGAC GGTACGTCTA CGACTTCCTC TGGTAGTTAC TCCTCCGACG

1901  TGAGTGGGAC AGGCTGCATC CTGTGCACGC TGGCCCCATT GCCCCCGGCC
      ACTCACCCTG TCCGACGTAG GACACGTGCG ACCGGGGGTAA CGGGGGCCGG

1951  AGATGAGGGA GCCCAGGGGC TCTGACATTG CTGGCACCAC CTCCACCCTC
      TCTACTCCCT CGGGTCCCCG AGACTGTAAC GACCGTGGTG GAGGTGGGAG

2001  CAGGAGCAGA TTGGCTGGAT GACCAACAAC CCCCCCATCC CTGTGGGGGA
      GTCCTCGTCT AACCGACCTA CTGGTTGTTG GGGGGGTAGG GACACCCCCT

2051  AATCTACAAG AGGTGGATCA TCCTGGGCCT GAACAAGATT GTGAGGATGT
      TTAGATGTTC TCCACCTAGT AGGACCCGGA CTTGTTCTAA CACTCCTACA

2101  ACTCCCCCAC CTCCATCCTG GACATCAGGC AGGGCCCCAA GGAGCCCTTC
      TGAGGGGGTG GAGGTAGGAC CTGTAGTCCG TCCCGGGGTT CCTCGGGAAG

2151  AGGGACTATG TGGACAGGTT CTACAAGACC CTGAGGGCTG AGCAGGCCTC
      TCCCTGATAC ACCTGTCCAA GATGTTCTGG GACTCCCGAC TCGTCCGGAG

2201  CCAGGAGGTG AAGAACTGGA TGACAGAGAC CCTGCTGGTG CAGAATGCCA
      GGTCCTCCAC TTCTTGACCT ACTGTCTCTG GGACGACCAC GTCTTACGGT

2251  ACCCTGACTG CAAGACCATC CTGAAGGCCC TGGGCCCTGC TGCCACCCTG
      TGGGACTGAC GTTCTGGTAG GACTTCCGGG ACCCGGGACG ACGGTGGGAC

2301  GAGGAGATGA TGACAGCCTG CCAGGGGGTG GGGGGCCCTG GTCACAAGGC
      CTCCTCTACT ACTGTCGGAC GGTCCCCCAC CCCCGGGGAC CAGTGTTCCG

2351  CAGGGTGCTG GCTGAGGCCA TGTCCCAGGT GACCAACTCC GCCACCATCA
      GTCCCACGAC CGACTCCGGT ACAGGGTCCA CTGGTTGAGG CGGTGGTAGT

2401  TGATGCAGAG GGGCAACTTC AGGAACCAGA GGAAGACAGT GAAGTGCTTC
      ACTACGTCTC CCCGTTGAAG TCCTTGGTCT CCTTCTGTCA CTTCACGAAG

2451  AACTGTGGCA AGGTGGGCCA CATTGCCAAG AACTGTAGGG CCCCCAGGAA
      TTGACACCGT TCCACCCGGT GTAACGGTTC TTGACATCCC GGGGGTCCTT
```

FIG.9A-3

2501 GAAGGGCTGC TGGAAGTGTG GCAAGGAGGG CCACCAGATG AAGGACTGCA
CTTCCCGACG ACCTTCACAC CGTTCCTCCC GGTGGTCTAC TTCCTGACGT

2551 ATGAGAGGCA GGCCAACTTC CTGGGCAAAA TCTGGCCCTC CCACAAGGGC
TACTCTCCGT CCGGTTGAAG GACCCGTTTT AGACCGGGAG GGTGTTCCCG

2601 AGGCCTGGCA ACTTCCTCCA GTCCAGGCCT GAGCCCACAG CCCCTCCCGA
TCCGGACCGT TGAAGGAGGT CAGGTCCGGA CTCGGGTGTC GGGGAGGGCT

2651 GGAGTCCTTC AGGTTTGGGG AGGAGAAGAC CACCCCCAGC CAGAAGCAGG
CCTCAGGAAG TCCAAACCCC TCCTCTTCTG GTGGGGGTCG GTCTTCGTCC

2701 AGCCCATTGA CAAGGAGCTG TACCCCCTGG CCTCCCTGAG GTCCCTGTTT
TCGGGTAACT GTTCCTCGAC ATGGGGGACC GGAGGGACTC CAGGGACAAA

2751 GGCAACGACC CCTCCTCCCA GTAAAATAAA GCCCGGGCAG ATCTGCTGTG
CCGTTGCTGG GGAGGAGGGT CATTTTATTT CGGGCCCGTC TAGACGACAC

2801 CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT
GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGGGC ACGGAAGGAA

2851 GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA
CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT TTACTCCTTT

2901 TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG
AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC CCCACCCCAC

2951 GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA GGCATGCTGG
CCCGTCCTGT CGTTCCCCCT CCTAACCCTT CTGTTATCGT CCGTACGACC

3001 GGATGCGGTG GGCTCTATGG CCGATCGGCG CGCCGTACTG AAATGTGTGG
CCTACGCCAC CCGAGATACC GGCTAGCCGC GCGGCATGAC TTTACACACC

3051 GCGTGGCTTA AGGGTGGGAA AGAATATATA AGGTGGGGGT CTTATGTAGT
CGCACCGAAT TCCCACCCTT TCTTATATAT TCCACCCCCA GAATACATCA

3101 TTTGTATCTG TTTTGCAGCA GCCGCCGCCG CCATGAGCAC CAACTCGTTT
AAACATAGAC AAAACGTCGT CGGCGGCGGC GGTACTCGTG GTTGAGCAAA

3151 GATGGAAGCA TTGTGAGCTC ATATTTGACA ACGCGCATGC CCCCATGGGC
CTACCTTCGT AACACTCGAG TATAAACTGT TGCGCGTACG GGGGTACCCG

3201 CGGGGTGCGT CAGAATGTGA TGGGCTCCAG CATTGATGGT CGCCCCGTCC
GCCCCACGCA GTCTTACACT ACCCGAGGTC GTAACTACCA GCGGGGCAGG

3251 TGCCCGCAAA CTCTACTACC TTGACCTACG AGACCGTGTC TGGAACGCCG
ACGGGCGTTT GAGATGATGG AACTGGATGC TCTGGCACAG ACCTTGCGGC

3301 TTGGAGACTG CAGCCTCCGC CGCCGCTTCA GCCGCTGCAG CCACCGCCCG
AACCTCTGAC GTCGGAGGCG GCGGCGAAGT CGGCGACGTC GGTGGCGGGC

FIG.9A-4

```
3351  CGGGATTGTG ACTGACTTTG CTTTCCTGAG CCCGCTTGCA AACAGTGCAG
      GCCCTAACAC TGACTGAAAC GAAAGGACTC GGGCGAACGT TTGTCACGTC

3401  CTTCCCGTTC ATCCGCCCGC GATGACAAGT TGACGGCTCT TTTGGCACAA
      GAAGGGCAAG TAGGCGGGCG CTACTGTTCA ACTGCCGAGA AAACCGTGTT

3451  TTGGATTCTT TGACCCGGGA ACTTAATGTC GTTTCTCAGC AGCTGTTGGA
      AACCTAAGAA ACTGGGCCCT TGAATTACAG CAAAGAGTCG TCGACAACCT

3501  TCTGCGCCAG CAGGTTTCTG CCCTGAAGGC TTCCTCCCCT CCCAATGCGG
      AGACGCGGTC GTCCAAAGAC GGGACTTCCG AAGGAGGGGA GGGTTACGCC

3551  TTTAAAACAT AAATAAAAAA CCAGACTCTG TTTGGATTTG GATCAAGCAA
      AAATTTTGTA TTTATTTTTT GGTCTGAGAC AAACCTAAAC CTAGTTCGTT

3601  GTGTCTTGCT GTCTTTATTT AGGGGTTTTG CGCGCGCGGT AGGCCCGGGA
      CACAGAACGA CAGAAATAAA TCCCCAAAAC GCGCGCGCCA TCCGGGCCCT

3651  CCAGCGGTCT CGGTCGTTGA GGGTCCTGTG TATTTTTTCC AGGACGTGGT
      GGTCGCCAGA GCCAGCAACT CCCAGGACAC ATAAAAAAGG TCCTGCACCA

3701  AAAGGTGACT CTGGATGTTC AGATACATGG GCATAAGCCC GTCTCTGGGG
      TTTCCACTGA GACCTACAAG TCTATGTACC CGTATTCGGG CAGAGACCCC

3751  TGGAGGTAGC ACCACTGCAG AGCTTCATGC TGCGGGGTGG TGTTGTAGAT
      ACCTCCATCG TGGTGACGTC TCGAAGTACG ACGCCCCACC ACAACATCTA

3801  GATCCAGTCG TAGCAGGAGC GCTGGGCGTG GTGCCTAAAA ATGTCTTTCA
      CTAGGTCAGC ATCGTCCTCG CGACCCGCAC CACGGATTTT TACAGAAAGT

3851  GTAGCAAGCT GATTGCCAGG GGCAGGCCCT TGGTGTAAGT GTTTACAAAG
      CATCGTTCGA CTAACGGTCC CCGTCCGGGA ACCACATTCA CAAATGTTTC

3901  CGGTTAAGCT GGGATGGGTG CATACGTGGG GATATGAGAT GCATCTTGGA
      GCCAATTCGA CCCTACCCAC GTATGCACCC CTATACTCTA CGTAGAACCT

3951  CTGTATTTTT AGGTTGGCTA TGTTCCCAGC CATATCCCTC CGGGGATTCA
      GACATAAAAA TCCAACCGAT ACAAGGGTCG GTATAGGGAG GCCCCTAAGT

4001  TGTTGTGCAG AACCACCAGC ACAGTGTATC CGGTGCACTT GGGAAATTTG
      ACAACACGTC TTGGTGGTCG TGTCACATAG GCCACGTGAA CCCTTTAAAC

4051  TCATGTAGCT TAGAAGGAAA TGCGTGGAAG AACTTGGAGA CGCCCTTGTG
      AGTACATCGA ATCTTCCTTT ACGCACCTTC TTGAACCTCT GCGGGAACAC

4101  ACCTCCAAGA TTTTCCATGC ATTCGTCCAT AATGATGGCA ATGGGCCCAC
      TGGAGGTTCT AAAAGGTACG TAAGCAGGTA TTACTACCGT TACCCGGGTG

4151  GGGCGGCGGC CTGGGCGAAG ATATTTCTGG GATCACTAAC GTCATAGTTG
      CCCGCCGCCG GACCCGCTTC TATAAAGACC CTAGTGATTG CAGTATCAAC
```

FIG.9A-5

```
4201  TGTTCCAGGA TGAGATCGTC ATAGGCCATT TTTACAAAGC GCGGGCGGAG
      ACAAGGTCCT ACTCTAGCAG TATCCGGTAA AAATGTTTCG CGCCCGCCTC

4251  GGTGCCAGAC TGCGGTATAA TGGTTCCATC CGGCCCAGGG GCGTAGTTAC
      CCACGGTCTG ACGCCATATT ACCAAGGTAG GCCGGGTCCC CGCATCAATG

4301  CCTCACAGAT TTGCATTTCC CACGCTTTGA GTTCAGATGG GGGGATCATG
      GGAGTGTCTA AACGTAAAGG GTGCGAAACT CAAGTCTACC CCCCTAGTAC

4351  TCTACCTGCG GGGCGATGAA GAAAACGGTT TCCGGGGTAG GGGAGATCAG
      AGATGGACGC CCCGCTACTT CTTTTGCCAA AGGCCCCATC CCCTCTAGTC

4401  CTGGGAAGAA AGCAGGTTCC TGAGCAGCTG CGACTTACCG CAGCCGGTGG
      GACCCTTCTT TCGTCCAAGG ACTCGTCGAC GCTGAATGGC GTCGGCCACC

4451  GCCCGTAAAT CACACCTATT ACCGGCTGCA ACTGGTAGTT AAGAGAGCTG
      CGGGCATTTA GTGTGGATAA TGGCCGACGT TGACCATCAA TTCTCTCGAC

4501  CAGCTGCCGT CATCCCTGAG CAGGGGGGCC ACTTCGTTAA GCATGTCCCT
      GTCGACGGCA GTAGGGACTC GTCCCCCCGG TGAAGCAATT CGTACAGGGA

4551  GACTCGCATG TTTTCCCTGA CCAAATCCGC CAGAAGGCGC TCGCCGCCCA
      CTGAGCGTAC AAAAGGGACT GGTTTAGGCG GTCTTCCGCG AGCGGCGGGT

4601  GCGATAGCAG TTCTTGCAAG GAAGCAAAGT TTTTCAACGG TTTGAGACCG
      CGCTATCGTC AAGAACGTTC CTTCGTTTCA AAAAGTTGCC AAACTCTGGC

4651  TCCGCCGTAG GCATGCTTTT GAGCGTTTGA CCAAGCAGTT CCAGGCGGTC
      AGGCGGCATC CGTACGAAAA CTCGCAAACT GGTTCGTCAA GGTCCGCCAG

4701  CCACAGCTCG GTCACCTGCT CTACGGCATC TCGATCCAGC ATATCTCCTC
      GGTGTCGAGC CAGTGGACGA GATGCCGTAG AGCTAGGTCG TATAGAGGAG

4751  GTTTCGCGGG TTGGGGCGGC TTTCGCTGTA CGGCAGTAGT CGGTGCTCGT
      CAAAGCGCCC AACCCCGCCG AAAGCGACAT GCCGTCATCA GCCACGAGCA

4801  CCAGACGGGC CAGGGTCATG TCTTTCCACG GGCGCAGGGT CCTCGTCAGC
      GGTCTGCCCG GTCCCAGTAC AGAAAGGTGC CCGCGTCCCA GGAGCAGTCG

4851  GTAGTCTGGG TCACGGTGAA GGGGTGCGCT CCGGGCTGCG CGCTGGCCAG
      CATCAGACCC AGTGCCACTT CCCCACGCGA GGCCCGACGC GCGACCGGTC

4901  GGTGCGCTTG AGGCTGGTCC TGCTGGTGCT GAAGCGCTGC CGGTCTTCGC
      CCACGCGAAC TCCGACCAGG ACGACCACGA CTTCGCGACG GCCAGAAGCG

4951  CCTGCGCGTC GGCCAGGTAG CATTTGACCA TGGTGTCATA GTCCAGCCCC
      GGACGCGCAG CCGGTCCATC GTAAACTGGT ACCACAGTAT CAGGTCGGGG

5001  TCCGCGGCGT GGCCCTTGGC GCGCAGCTTG CCCTTGGAGG AGGCGCCGCA
      AGGCGCCGCA CCGGGAACCG CGCGTCGAAC GGGAACCTCC TCCGCGGCGT
```

FIG.9A-6

```
5051  CGAGGGGCAG TGCAGACTTT TGAGGGCGTA GAGCTTGGGC GCGAGAAATA
      GCTCCCCGTC ACGTCTGAAA ACTCCCGCAT CTCGAACCCG CGCTCTTTAT

5101  CCGATTCCGG GGAGTAGGCA TCCGCGCCGC AGGCCCCGCA GACGGTCTCG
      GGCTAAGGCC CCTCATCCGT AGGCGCGGCG TCCGGGGCGT CTGCCAGAGC

5151  CATTCCACGA GCCAGGTGAG CTCTGGCCGT TCGGGGTCAA AAACCAGGTT
      GTAAGGTGCT CGGTCCACTC GAGACCGGCA AGCCCCAGTT TTTGGTCCAA

5201  TCCCCCATGC TTTTTGATGC GTTTCTTACC TCTGGTTTCC ATGAGCCGGT
      AGGGGGTACG AAAAACTACG CAAAGAATGG AGACCAAAGG TACTCGGCCA

5251  GTCCAGCGTC GGTGACGAAA AGGCTGTCCG TGTCCCCGTA TACAGACTTG
      CAGGTGCGAG CCACTGCTTT TCCGACAGGC ACAGGGGCAT ATGTCTGAAC

5301  AGAGGCCTGT CCTCGAGCGG TGTTCCGCGG TCCTCCTCGT ATAGAAACTC
      TCTCCGGACA GGAGCTCGCC ACAAGGCGCC AGGAGGAGCA TATCTTTGAG

5351  GGACCACTCT GAGACAAAGG CTCGCGTCCA GGCCAGCACG AAGGAGGCTA
      CCTGGTGAGA CTCTGTTTCC GAGCGCAGGT CCGGTCGTGC TTCCTCCGAT

5401  AGTGGGAGGG GTAGCGGTCG TTGTCCACTA GGGGGTCCAC TCGCTCCAGG
      TCACCCTCCC CATCGCCAGC AACAGGTGAT CCCCCAGGTG AGCGAGGTCC

5451  GTGTGAAGAC ACATGTCGCC CTCTTCGGCA TCAAGGAAGG TGATTGGTTT
      CACACTTCTG TGTACAGCGG GAGAAGCCGT AGTTCCTTCC ACTAACCAAA

5501  GTAGGTGTAG GCCACGTGAC CGGGTGTTCC TGAAGGGGGG CTATAAAAGG
      CATCCACATC CGGTGCACTG GCCCACAAGG ACTTCCCCCC GATATTTTCC

5551  GGGTGGGGGC GCGTTCGTCC TCACTCTCTT CCGCATCGCT GTCTGCGAGG
      CCCACCCCCG CGCAAGCAGG AGTGAGAGAA GGCGTAGCGA CAGACGCTCC

5601  GCCAGCTGTT GGGGTGAGTA CTCCCTCTGA AAAGCGGGCA TGACTTCTGC
      CGGTCGACAA CCCCACTCAT GAGGGAGACT TTTCGCCCGT ACTGAAGACG

5651  GCTAAGATTG TCAGTTTCCA AAAACGAGGA GGATTTGATA TTCACCTGGC
      CGATTCTAAC AGTCAAAGGT TTTTGCTCCT CCTAAACTAT AAGTGGACCG

5701  CCGCGGTGAT GCCTTTGAGG GTGGCCGCAT CCATCTGGTC AGAAAAGACA
      GGCGCCACTA CGGAAACTCC CACCGGCGTA GGTAGACCAG TCTTTTCTGT

5751  ATCTTTTTGT TGTCAAGCTT GGTGGCAAAC GACCCGTAGA GGGCGTTGGA
      TAGAAAAACA ACAGTTCGAA CCACCGTTTG CTGGGCATCT CCCGCAACCT

5801  CAGCAACTTG GCGATGGAGC GCAGGGTTTG GTTTTTGTCG CGATCGGCGC
      GTCGTTGAAC CGCTACCTCG CGTCCCAAAC CAAAAACAGC GCTAGCCGCG

5851  GCTCCTTGGC CGCGATGTTT AGCTGCACGT ATTCGCGCGC AACGCACCGC
      CGAGGAACCG GCGCTACAAA TCGACGTGCA TAAGCGCGCG TTGCGTGGCG
```

FIG.9A-7

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5901  CATTCGGGAA AGACGGTGGT GCGCTCGTCG GGCACCAGGT GCACGCGCCA
      GTAAGCCCTT TCTGCCACCA CGCGAGCAGC CCGTGGTCCA CGTGCGCGGT

5951  ACCGCGGTTG TGCAGGGTGA CAAGGTCAAC GCTGGTGGCT ACCTCTCCGC
      TGGCGCCAAC ACGTCCCACT GTTCCAGTTG CGACCACCGA TGGAGAGGCG

6001  GTAGGCGCTC GTTGGTCCAG CAGAGGCGGC CGCCCTTGCG CGAGCAGAAT
      CATCCGCGAG CAACCAGGTC GTCTCCGCCG GCGGGAACGC GCTCGTCTTA

6051  GGCGGTAGGG GGTCTAGCTG CGTCTCGTCC GGGGGGTCTG CGTCCACGGT
      CCGCCATCCC CCAGATCGAC GCAGAGCAGG CCCCCCAGAC GCAGGTGCCA

6101  AAAGACCCCG GGCAGCAGGC GCGCGTCGAA GTAGTCTATC TTGCATCCTT
      TTTCTGGGGC CCGTCGTCCG CGCGCAGCTT CATCAGATAG AACGTAGGAA

6151  GCAAGTCTAG CGCCTGCTGC CATGCGCGGG CGGCAAGCGC GCGCTCGTAT
      CGTTCAGATC GCGGACGACG GTACGCGCCC GCCGTTCGCG CGCGAGCATA

6201  GGGTTGAGTG GGGGACCCCA TGGCATGGGG TGGGTGAGCG CGGAGGCGTA
      CCCAACTCAC CCCCTGGGGT ACCGTACCCC ACCCACTCGC GCCTCCGCAT

6251  CATGCCGCAA ATGTCGTAAA CGTAGAGGGG CTCTCTGAGT ATTCCAAGAT
      GTACGGCGTT TACAGCATTT GCATCTCCCC GAGAGACTCA TAAGGTTCTA

6301  ATGTAGGGTA GCATCTTCCA CCGCGGATGC TGGCGCGCAC GTAATCGTAT
      TACATCCCAT CGTAGAAGGT GGCGCCTACG ACCGCGCGTG CATTAGCATA

6351  AGTTCGTGCG AGGGAGCGAG GAGGTCGGGA CCGAGGTTGC TACGGGCGGG
      TCAAGCACGC TCCCTCGCTC CTCCAGCCCT GGCTCCAACG ATGCCCGCCC

6401  CTGCTCTGCT CGGAAGACTA TCTGCCTGAA GATGGCATGT GAGTTGGATG
      GACGAGACGA GCCTTCTGAT AGACGGACTT CTACCGTACA CTCAACCTAC

6451  ATATGGTTGG ACGCTGGAAG ACGTTGAAGC TGGCGTCTGT GAGACCTACC
      TATACCAACC TGCGACCTTC TGCAACTTCG ACCGCAGACA CTCTGGATGG

6501  GCGTCACGCA CGAAGGAGGC GTAGGAGTCG CGCAGCTTGT TGACCAGCTC
      CGCAGTGCGT GCTTCCTCCG CATCCTCAGC GCGTCGAACA ACTGGTCGAG

6551  GGCGGTGACC TGCACGTCTA GGGCGCAGTA GTCCAGGGTT TCCTTGATGA
      CCGCCACTGG ACGTGCAGAT CCCGCGTCAT CAGGTCCCAA AGGAACTACT

6601  TGTCATACTT ATCCTGTCCC TTTTTTTTCC ACAGCTCGCG GTTGAGGACA
      ACAGTATGAA TAGGACAGGG AAAAAAAAGG TGTCGAGCGC CAACTCCTGT

6651  AACTCTTCGC GGTCTTTCCA GTACTCTTGG ATCGGAAACC CGTCGGCCTC
      TTGAGAAGCG CCAGAAAGGT CATGAGAACC TAGCCTTTGG GCAGCCGGAG

6701  CGAACGGTAA GAGCCTAGCA TGTAGAACTG GTTGACGGCC TGGTAGGCGC
      GCTTGCCATT CTCGGATCGT ACATCTTGAC CAACTGCCGG ACCATCCGCG
```

FIG.9A-8

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6751  AGCATCCCTT TTCTACGGGT AGCGCGTATG CCTGCGCGGC CTTCCGGAGC
      TCGTAGGGAA AAGATGCCCA TCGCGCATAC GGACGCGCCG GAAGGCCTCG

6801  GAGGTGTGGG TGAGCGCAAA GGTGTCCCTG ACCATGACTT TGAGGTACTG
      CTCCACACCC ACTCGCGTTT CCACAGGGAC TGGTACTGAA ACTCCATGAC

6851  GTATTTGAAG TCAGTGTCGT CGCATCCGCC CTGCTCCCAG AGCAAAAAGT
      CATAAACTTC AGTCACAGCA GCGTAGGCGG GACGAGGGTC TCGTTTTTCA

6901  CCGTGCGCTT TTTGGAACGC GGATTTGGCA GGGCGAAGGT GACATCGTTG
      GGCACGCGAA AAACCTTGCG CCTAAACCGT CCCGCTTCCA CTGTAGCAAC

6951  AAGAGTATCT TTCCCGCGCG AGGCATAAAG TTGCGTGTGA TGCGGAAGGG
      TTCTCATAGA AAGGGCGCGC TCCGTATTTC AACGCACACT ACGCCTTCCC

7001  TCCCGGCACC TCGGAACGGT TGTTAATTAC CTGGGCGGCG AGCACGATCT
      AGGGCCGTGG AGCCTTGCCA ACAATTAATG GACCCGCCGC TCGTGCTAGA

7051  CGTCAAAGCC GTTGATGTTG TGGCCCACAA TGTAAAGTTC CAAGAAGCGC
      GCAGTTTCGG CAACTACAAC ACCGGGTGTT ACATTTCAAG GTTCTTCGCG

7101  GGGATGCCCT TGATGGAAGG CAATTTTTTA AGTTCCTCGT AGGTGAGCTC
      CCCTACGGGA ACTACCTTCC GTTAAAAAAT TCAAGGAGCA TCCACTCGAG

7151  TTCAGGGGAG CTGAGCCCGT GCTCTGAAAG GGCCCAGTCT GCAAGATGAG
      AAGTCCCCTC GACTCGGGCA CGAGACTTTC CCGGGTCAGA CGTTCTACTC

7201  GGTTGGAAGC GACGAATGAG CTCCACAGGT CACGGGCCAT TAGCATTTGC
      CCAACCTTCG CTGCTTACTC GAGGTGTCCA GTGCCCGGTA ATCGTAAACG

7251  AGGTGGTCGC GAAAGGTCCT AAACTGGCGA CCTATGGCCA TTTTTTCTGG
      TCCACCAGCG CTTTCCAGGA TTTGACCGCT GGATACCGGT AAAAAAGACC

7301  GGTGATGCAG TAGAAGGTAA GCGGGTCTTG TTCCCAGCGG TCCCATCCAA
      CCACTACGTC ATCTTCCATT CGCCCAGAAC AAGGGTCGCC AGGGTAGGTT

7351  GGTTCGCGGC TAGGTCTCGC GCGGCAGTCA CTAGAGGCTC ATCTCCGCCG
      CCAAGCGCCG ATCCAGAGCG CGCCGTCAGT GATCTCCGAG TAGAGGCGGC

7401  AACTTCATGA CCAGCATGAA GGGCACGAGC TGCTTCCCAA AGGCCCCCAT
      TTGAAGTACT GGTCGTACTT CCCGTGCTCG ACGAAGGGTT TCCGGGGGTA

7451  CCAAGTATAG GTCTCTACAT CGTAGGTGAC AAAGAGACGC TCGGTGCGAG
      GGTTCATATC CAGAGATGTA GCATCCACTG TTTCTCTGCG AGCCACGCTC

7501  GATGCGAGCC GATCGGGAAG AACTGGATCT CCCGCCACCA ATTGGAGGAG
      CTACGCTCGG CTAGCCCTTC TTGACCTAGA GGGCGGTGGT TAACCTCCTC

7551  TGGCTATTGA TGTGGTGAAA GTAGAAGTCC CTGCGACGGG CCGAACACTC
      ACCGATAACT ACACCACTTT CATCTTCAGG GACGCTGCCC GGCTTGTGAG
```

FIG.9A-9

```
7601  GTGCTGGCTT TTGTAAAAAC GTGCGCAGTA CTGGCAGCGG TGCACGGGCT
      CACGACCGAA AACATTTTTG CACGCGTCAT GACCGTCGCC ACGTGCCCGA

7651  GTACATCCTG CACGAGGTTG ACCTGACGAC CGCGCACAAG GAAGCAGAGT
      CATGTAGGAC GTGCTCCAAC TGGACTGCTG GCGCGTGTTC CTTCGTCTCA

7701  GGGAATTTGA GCCCCTCGCC TGGCGGGTTT GGCTGGTGGT CTTCTACTTC
      CCCTTAAACT CGGGGAGCGG ACCGCCCAAA CCGACCACCA GAAGATGAAG

7751  GGCTGCTTGT CCTTGACCGT CTGGCTGCTC GAGGGGAGTT ACGGTGGATC
      CCGACGAACA GGAACTGGCA GACCGACGAG CTCCCCTCAA TGCCACCTAG

7801  GGACCACCAC GCCGCGCGAG CCCAAAGTCC AGATGTCCGC GCGCGGCGGT
      CCTGGTGGTG CGGCGCGCTC GGGTTTCAGG TCTACAGGCG CGCGCCGCCA

7851  CGGAGCTTGA TGACAACATC GCGCAGATGG GAGCTGTCCA TGGTCTGGAG
      GCCTCGAACT ACTGTTGTAG CGCGTCTACC CTCGACAGGT ACCAGACCTC

7901  CTCCCGCGGC GTCAGGTCAG GCGGGAGCTC CTGCAGGTTT ACCTCGCATA
      GAGGGCGCCG CAGTCCAGTC CGCCCTCGAG GACGTCCAAA TGGAGCGTAT

7951  GACGGGTCAG GGCGCGGGCT AGATCCAGGT GATACCTAAT TTCCAGGGGC
      CTGCCCAGTC CCGCGCCCGA TCTAGGTCCA CTATGGATTA AAGGTCCCCG

8001  TGGTTGGTGG CGGCGTCGAT GGCTTGCAAG AGGCCGCATC CCCGCGGCGC
      ACCAACCACC GCCGCAGCTA CCGAACGTTC TCCGGCGTAG GGGCGCCGCG

8051  GACTACGGTA CCGCGCGGCG GGCGGTGGGC CGCGGGGGTG TCCTTGGATG
      CTGATGCCAT GGCGCGCCGC CCGCCACCCG GCGCCCCCAC AGGAACCTAC

8101  ATGCATCTAA AAGCGGTGAC GCGGGCGAGC CCCCGGAGGT AGGGGGGGCT
      TACGTAGATT TTCGCCACTG CGCCCGCTCG GGGGCCTCCA TCCCCCCGA

8151  CCGGACCCGC CGGGAGAGGG GGCAGGGGCA CGTCGGCGCC GCGCGCGGGC
      GGCCTGGGCG GCCCTCTCCC CCGTCCCCGT GCAGCCGCGG CGCGCGCCCG

8201  AGGAGCTGGT GCTGCGCGCG TAGGTTGCTG GCGAACGCGA CGACGCGGCG
      TCCTCGACCA CGACGCGCGC ATCCAACGAC CGCTTGCGCT GCTGCGCCGC

8251  GTTGATCTCC TGAATCTGGC GCCTCTGCGT GAAGACGACG GGCCCGGTGA
      CAACTAGAGG ACTTAGACCG CGGAGACGCA CTTCTGCTGC CCGGGCCACT

8301  GCTTGAACCT GAAAGAGAGT TCGACAGAAT CAATTTCGGT GTCGTTGACG
      CGAACTTGGA CTTTCTCTCA AGCTGTCTTA GTTAAAGCCA CAGCAACTGC

8351  GCGGCCTGGC GCAAAATCTC CTGCACGTCT CCTGAGTTGT CTTGATAGGC
      CGCCGGACCG CGTTTTAGAG GACGTGCAGA GGACTCAACA GAACTATCCG

8401  GATCTCGGCC ATGAACTGCT CGATCTCTTC CTCCTGGAGA TCTCCGCGTC
      CTAGAGCCGG TACTTGACGA GCTAGAGAAG GAGGACCTCT AGAGGCGCAG
```

FIG.9A-10

8451 CGGCTCGCTC CACGGTGGCG GCGAGGTCGT TGGAAATGCG GGCCATGAGC
GCCGAGCGAG GTGCCACCGC CGCTCCAGCA ACCTTTACGC CCGGTACTCG

8501 TGCGAGAAGG CGTTGAGGCC TCCCTCGTTC CAGACGCGGC TGTAGACCAC
ACGCTCTTCC GCAACTCCGG AGGGAGCAAG GTCTGCGCCG ACATCTGGTG

8551 GCCCCCTTCG GCATCGCGGG CGCGCATGAC CACCTGCGCG AGATTGAGCT
CGGGGGAAGC CGTAGCGCCC GCGCGTACTG GTGGACGCGC TCTAACTCGA

8601 CCACGTGCCG GGCGAAGACG GCGTAGTTTC GCAGGCGCTG AAAGAGGTAG
GGTGCACGGC CCGCTTCTGC CGCATCAAAG CGTCCGCGAC TTTCTCCATC

8651 TTGAGGGTGG TGGCGGTGTG TTCTGCCACG AAGAAGTACA TAACCCAGCG
AACTCCCACC ACCGCCACAC AAGACGGTGC TTCTTCATGT ATTGGGTCGC

8701 TCGCAACGTG GATTCGTTGA TATCCCCCAA GGCCTCAAGG CGCTCCATGG
AGCGTTGCAC CTAAGCAACT ATAGGGGGTT CCGGAGTTCC GCGAGGTACC

8751 CCTCGTAGAA GTCCACGGCG AAGTTGAAAA ACTGGGAGTT GCGCGCCGAC
GGAGCATCTT CAGGTGCCGC TTCAACTTTT TGACCCTCAA CGCGCGGCTG

8801 ACGGTTAACT CCTCCTCCAG AAGACGGATG AGCTCGGCGA CAGTGTCGCG
TGCCAATTGA GGAGGAGGTC TTCTGCCTAC TCGAGCCGCT GTCACAGCGC

8851 CACCTCGCGC TCAAAGGCTA CAGGGGCCTC TTCTTCTTCT TCAATCTCCT
GTGGAGCGCG AGTTTCCGAT GTCCCCGGAG AAGAAGAAGA AGTTAGAGGA

8901 CTTCCATAAG GGCCTCCCCT TCTTCTTCTT CTGGCGGCGG TGGGGGAGGG
GAAGGTATTC CCGGAGGGGA AGAAGAAGAA GACCGCCGCC ACCCCCTCCC

8951 GGGACACGGC GGCGACGACG GCGCACCGGG AGGCGGTCGA CAAAGCGCTC
CCCTGTGCCG CCGCTGCTGC CGCGTGGCCC TCCGCCAGCT GTTTCGCGAG

9001 GATCATCTCC CCGCGGCGAC GGCGCATGGT CTCGGTGACG GCGCGGCCGT
CTAGTAGAGG GGCGCCGCTG CCGCGTACCA GAGCCACTGC CGCGCCGGCA

9051 TCTCGCGGGG GCGCAGTTGG AAGACGCCGC CCGTCATGTC CCGGTTATGG
AGAGCGCCCC CGCGTCAACC TTCTGCGGCG GGCAGTACAG GGCCAATACC

9101 GTTGGCGGGG GGCTGCCATG CGGCAGGGAT ACGGCGCTAA CGATGCATCT
CAACCGCCCC CCGACGGTAC GCCGTCCCTA TGCCGCGATT GCTACGTAGA

9151 CAACAATTGT TGTGTAGGTA CTCCGCCGCC GAGGGACCTG AGCGAGTCCG
GTTGTTAACA ACACATCCAT GAGGCGGCGG CTCCCTGGAC TCGCTCAGGC

9201 CATCGACCGG ATCGGAAAAC CTCTCGAGAA AGGCGTCTAA CCAGTCACAG
GTAGCTGGCC TAGCCTTTTG GAGAGCTCTT TCCGCAGATT GGTCAGTGTC

9251 TCGCAAGGTA GGCTGAGCAC CGTGGCGGGC GGCAGCGGGC GGCGGTCGGG
AGCGTTCCAT CCGACTCGTG GCACCGCCCG CCGTCGCCCG CCGCCAGCCC

FIG.9A-11

```
9301  GTTGTTTCTG GCGGAGGTGC TGCTGATGAT GTAATTAAAG TAGGCGGTCT
      CAACAAAGAC CGCCTCCACG ACGACTACTA CATTAATTTC ATCCGCCAGA

9351  TGAGACGGCG GATGGTCGAC AGAAGCACCA TGTCCTTGGG TCCGGCCTGC
      ACTCTGCCGC CTACCAGCTG TCTTCGTGGT ACAGGAACCC AGGCCGGACG

9401  TGAATGCGCA GGCGGTCGGC CATGCCCCAG GCTTCGTTTT GACATCGGCG
      ACTTACGCGT CCGCCAGCCG GTACGGGGTC CGAAGCAAAA CTGTAGCCGC

9451  CAGGTCTTTG TAGTAGTCTT GCATGAGCCT TTCTACCGGC ACTTCTTCTT
      GTCCAGAAAC ATCATCAGAA CGTACTCGGA AAGATGGCCG TGAAGAAGAA

9501  CTCCTTCCTC TTGTCCTGCA TCTCTTGCAT CTATCGCTGC GGCGGCGGCG
      GAGGAAGGAG AACAGGACGT AGAGAACGTA GATAGCGACG CCGCCGCCGC

9551  GAGTTTGGCC GTAGGTGGCG CCCTCTTCCT CCCATGCGTG TGACCCCGAA
      CTCAAACCGG CATCCACCGC GGGAGAAGGA GGGTACGCAC ACTGGGGCTT

9601  GCCCCTCATC GGCTGAAGCA GGGCTAGGTC GGCGACAACG CGCTCGGCTA
      CGGGGAGTAG CCGACTTCGT CCCGATCCAG CCGCTGTTGC GCGAGCCGAT

9651  ATATGGCCTG CTGCACCTGC GTGAGGGTAG ACTGGAAGTC ATCCATGTCC
      TATACCGGAC GACGTGGACG CACTCCCATC TGACCTTCAG TAGGTACAGG

9701  ACAAAGCGGT GGTATGCGCC CGTGTTGATG GTGTAAGTGC AGTTGGCCAT
      TGTTTCGCCA CCATACGCGG GCACAACTAC CACATTCACG TCAACCGGTA

9751  AACGGACCAG TTAACGGTCT GGTGACCCGG CTGCGAGAGC TCGGTGTACC
      TTGCCTGGTC AATTGCCAGA CCACTGGGCC GACGCTCTCG AGCCACATGG

9801  TGAGACGCGA GTAAGCCCTC GAGTCAAATA CGTAGTCGTT GCAAGTCCGC
      ACTCTGCGCT CATTCGGGAG CTCAGTTTAT GCATCAGCAA CGTTCAGGCG

9851  ACCAGGTACT GGTATCCCAC CAAAAAGTGC GGCGGCGGCT GGCGGTAGAG
      TGGTCCATGA CCATAGGGTG GTTTTTCACG CCGCCGCCGA CCGCCATCTC

9901  GGGCCAGCGT AGGGTGGCCG GGGCTCCGGG GGCGAGATCT TCCAACATAA
      CCCGGTCGCA TCCCACCGGC CCCGAGGCCC CCGCTCTAGA AGGTTGTATT

9951  GGCGATGATA TCCGTAGATG TACCTGGACA TCCAGGTGAT GCCGGCGGCG
      CCGCTACTAT AGGCATCTAC ATGGACCTGT AGGTCCACTA CGGCCGCCGC

10001 GTGGTGGAGG CGCGCGGAAA GTCGCGGACG CGGTTCCAGA TGTTGCGCAG
      CACCACCTCC GCGCGCCTTT CAGCGCCTGC GCCAAGGTCT ACAACGCGTC

10051 CGGCAAAAAG TGCTCCATGG TCGGGACGCT CTGGCCGGTC AGGCGCGCGC
      GCCGTTTTTC ACGAGGTACC AGCCCTGCGA GACCGGCCAG TCCGCGCGCG

10101 AATCGTTGAC GCTCTAGACC GTGCAAAAGG AGAGCCTGTA AGCGGGCACT
      TTAGCAACTG CGAGATCTGG CACGTTTTCC TCTCGGACAT TCGCCCGTGA
```

FIG.9A-12

```
10151  CTTCCGTGGT CTGGTGGATA AATTCGCAAG GGTATCATGG CGGACGACCG
       GAAGGCACCA GACCACCTAT TTAAGCGTTC CCATAGTACC GCCTGCTGGC

10201  GGGTTCGAGC CCCGTATCCG GCCGTCCGCC GTGATCCATG CGGTTACCGC
       CCCAAGCTCG GGGCATAGGC CGGCAGGCGG CACTAGGTAC GCCAATGGCG

10251  CCGCGTGTCG AACCCAGGTG TGCGACGTCA GACAACGGGG GAGTGCTCCT
       GGCGCACAGC TTGGGTCCAC ACGCTGCAGT CTGTTGCCCC CTCACGAGGA

10301  TTTTGGCTTCC TTCCAGGCGC GGCGGCTGCT GCGCTAGCTT TTTTGGCCAC
       AAACCGAAGG AAGGTCCGCG CCGCCGACGA CGCGATCGAA AAAACCGGTG

10351  TGGCCGCGCG CAGCGTAAGC GGTTAGGCTG GAAAGCGAAA GCATTAAGTG
       ACCGGCGCGC GTCGCATTCG CCAATCCGAC CTTTCGCTTT CGTAATTCAC

10401  GCTCGCTCCC TGTAGCCGGA GGGTTATTTT CCAAGGGTTG AGTCGCGGGA
       CGAGCGAGGG ACATCGGCCT CCCAATAAAA GGTTCCCAAC TCAGCGCCCT

10451  CCCCCGGTTC GAGTCTCGGA CCGGCCGGAC TGCGGCGAAC GGGGGTTTGC
       GGGGGCCAAG CTCAGAGCCT GGCCGGCCTG ACGCCGCTTG CCCCCAAACG

10501  CTCCCCGTCA TGCAAGACCC CGCTTGCAAA TTCCTCCGGA AACAGGGACG
       GAGGGGCAGT ACGTTCTGGG GCGAACGTTT AAGGAGGCCT TTGTCCCTGC

10551  AGCCCCTTTT TTGCTTTTCC CAGATGCATC CGGTGCTGCG GCAGATGCGC
       TCGGGGAAAA AACGAAAAGG GTCTACGTAG GCCACGACGC CGTCTACGCG

10601  CCCCCTCCTC AGCAGCGGCA AGAGCAAGAG CAGCGGCAGA CATGCAGGGC
       GGGGGAGGAG TCGTCGCCGT TCTCGTTCTC GTCGCCGTCT GTACGTCCCG

10651  ACCCTCCCCT CCTCCTACCG CGTCAGGAGG GGCGACATCC GCGGTTGACG
       TGGGAGGGGA GGAGGATGGC GCAGTCCTCC CCGCTGTAGG CGCCAACTGC

10701  CGGCAGCAGA TGGTGATTAC GAACCCCCGC GGCGCCGGGC CCGGCACTAC
       GCCGTCGTCT ACCACTAATG CTTGGGGGCG CCGCGGCCCG GGCCGTGATG

10751  CTGGACTTGG AGGAGGGCGA GGGCCTGGCG CGGCTAGGAG CGCCCTCTCC
       GACCTGAACC TCCTCCCGCT CCCGGACCGC GCCGATCCTC GCGGGAGAGG

10801  TGAGCGGCAC CCAAGGGTGC AGCTGAAGCG TGATACGCGT GAGGCGTACG
       ACTCGCCGTG GGTTCCCACG TCGACTTCGC ACTATGCGCA CTCCGCATGC

10851  TGCCGCGGCA GAACCTGTTT CGCGACCGCG AGGGAGAGGA GCCCGAGGAG
       ACGGCGCCGT CTTGGACAAA GCGCTGGCGC TCCCTCTCCT CGGGCTCCTC

10901  ATGCGGGATC GAAAGTTCCA CGCAGGGCGC GAGCTGCGGC ATGGCCTGAA
       TACGCCCTAG CTTTCAAGGT GCGTCCCGCG CTCGACGCCG TACCGGACTT

10951  TCGCGAGCGG TTGCTGCGCG AGGAGGACTT TGAGCCCGAC GCGCGAACCG
       AGCGCTCGCC AACGACGCGC TCCTCCTGAA ACTCGGGCTG CGCGCTTGGC
```

FIG.9A-13

```
11001  GGATTAGTCC CGCGCGCGCA CACGTGGCGG CCGCCGACCT GGTAACCGCA
       CCTAATCAGG GCGCGCGCGT GTGCACCGCC GGCGGCTGGA CCATTGGCGT

11051  TACGAGCAGA CGGTGAACCA GGAGATTAAC TTTCAAAAAA GCTTTAACAA
       ATGCTCGTCT GCCACTTGGT CCTCTAATTG AAAGTTTTTT CGAAATTGTT

11101  CCACGTGCGT ACGCTTGTGG CGCGCGAGGA GGTGGCTATA GGACTGATGC
       GGTGCACGCA TGCGAACACC GCGCGCTCCT CCACCGATAT CCTGACTACG

11151  ATCTGTGGGA CTTTGTAAGC GCGCTGGAGC AAAACCCAAA TAGCAAGCCG
       TAGACACCCT GAAACATTCG CGCGACCTCG TTTTGGGTTT ATCGTTCGGC

11201  CTCATGGCGC AGCTGTTCCT TATAGTGCAG CACAGCAGGG ACAACGAGGC
       GAGTACCGCG TCGACAAGGA ATATCACGTC GTGTCGTCCC TGTTGCTCCG

11251  ATTCAGGGAT GCGCTGCTAA ACATAGTAGA GCCCGAGGGC CGCTGGCTGC
       TAAGTCCCTA CGCGACGATT TGTATCATCT CGGGCTCCCG GCGACCGACG

11301  TCGATTTGAT AAACATCCTG CAGAGCATAG TGGTGCAGGA GCGCAGCTTG
       AGCTAAACTA TTTGTAGGAC GTCTCGTATC ACCACGTCCT CGCGTCGAAC

11351  AGCCTGGCTG ACAAGGTGGC CGCCATCAAC TATTCCATGC TTAGCCTGGG
       TCGGACCGAC TGTTCCACCG GCGGTAGTTG ATAAGGTACG AATCGGACCC

11401  CAAGTTTTAC GCCCGCAAGA TATACCATAC CCCTTACGTT CCCATAGACA
       GTTCAAAATG CGGGCGTTCT ATATGGTATG GGGAATGCAA GGGTATCTGT

11451  AGGAGGTAAA GATCGAGGGG TTCTACATGC GCATGGCGCT GAAGGTGCTT
       TCCTCCATTT CTAGCTCCCC AAGATGTACG CGTACCGCGA CTTCCACGAA

11501  ACCTTGAGCG ACGACCTGGG CGTTTATCGC AACGAGCGCA TCCACAAGGC
       TGGAACTCGC TGCTGGACCC GCAAATAGCG TTGCTCGCGT AGGTGTTCCG

11551  CGTGAGCGTG AGCCGGCGGC GCGAGCTCAG CGACCGCGAG CTGATGCACA
       GCACTCGCAC TCGGCCGCCG CGCTCGAGTC GCTGGCGCTC GACTACGTGT

11601  GCCTGCAAAG GGCCCTGGCT GGCACGGGCA GCGGCGATAG AGAGGCCGAG
       CGGACGTTTC CCGGGACCGA CCGTGCCCGT CGCCGCTATC TCTCCGGCTC

11651  TCCTACTTTG ACGCGGGCGC TGACCTGCGC TGGGCCCCAA GCCGACGCGC
       AGGATGAAAC TGCGCCCGCG ACTGGACGCG ACCCGGGGTT CGGCTGCGCG

11701  CCTGGAGGCA GCTGGGGCCG GACCTGGGCT GGCGGTGGCA CCCGCGCGCG
       GGACCTCCGT CGACCCCGGC CTGGACCCGA CCGCCACCGT GGGCGCGCGC

11751  CTGGCAACGT CGGCGGCGTG GAGGAATATG ACGAGGACGA TGAGTACGAG
       GACCGTTGCA GCCGCCGCAC CTCCTTATAC TGCTCCTGCT ACTCATGCTC

11801  CCAGAGGACG GCGAGTACTA AGCGGTGATG TTTCTGATCA GATGATGCAA
       GGTCTCCTGC CGCTCATGAT TCGCCACTAC AAAGACTAGT CTACTACGTT
```

FIG.9A-14

```
11851  GACGCAACGG ACCCGGCGGT GCGGGCGGCG CTGCAGAGCC AGCCGTCCGG
       CTGCGTTGCC TGGGCCGCCA CGCCCGCCGC GACGTCTCGG TCGGCAGGCC

11901  CCTTAACTCC ACGGACGACT GGCGCCAGGT CATGGACCGC ATCATGTCGC
       GGAATTGAGG TGCCTGCTGA CCGCGGTCCA GTACCTGGCG TAGTACAGCG

11951  TGACTGCGCG CAATCCTGAC GCGTTCCGGC AGCAGCCGCA GGCCAACCGG
       ACTGACGCGC GTTAGGACTG CGCAAGGCCG TCGTCGGCGT CCGGTTGGCC

12001  CTCTCCGCAA TTCTGGAAGC GGTGGTCCCG GCGCGCGCAA ACCCCACGCA
       GAGAGGCGTT AAGACCTTCG CCACCAGGGC CGCGCGCGTT TGGGGTGCGT

12051  CGAGAAGGTG CTGGCGATCG TAAACGCGCT GGCCGAAAAC AGGGCCATCC
       GCTCTTCCAC GACCGCTAGC ATTTGCGCGA CCGGCTTTTG TCCCGGTAGG

12101  GGCCCGACGA GGCCGGCCTG GTCTACGACG CGCTGCTTCA GCGCGTGGCT
       CCGGGCTGCT CCGGCCGGAC CAGATGCTGC GCGACGAAGT CGCGCACCGA

12151  CGTTACAACA GCGGCAACGT GCAGACCAAC CTGGACCGGC TGGTGGGGGA
       GCAATGTTGT CGCCGTTGCA CGTCTGGTTG GACCTGGCCG ACCACCCCCT

12201  TGTGCGCGAG GCCGTGGCGC AGCGTGAGCG CGCGCAGCAG CAGGGCAACC
       ACACGCGCTC CGGCACCGCG TCGCACTCGC GCGCGTCGTC GTCCCGTTGG

12251  TGGGCTCCAT GGTTGCACTA AACGCCTTCC TGAGTACACA GCCCGCCAAC
       ACCCGAGGTA CCAACGTGAT TTGCGGAAGG ACTCATGTGT CGGGCGGTTG

12301  GTGCCGCGGG GACAGGAGGA CTACACCAAC TTTGTGAGCG CACTGCGGCT
       CACGGCGCCC CTGTCCTCCT GATGTGGTTG AAACACTCGC GTGACGCCGA

12351  AATGGTGACT GAGACACCGC AAAGTGAGGT GTACCAGTCT GGGCCAGACT
       TTACCACTGA CTCTGTGGCG TTTCACTCCA CATGGTCAGA CCCGGTCTGA

12401  ATTTTTTCCA GACCAGTAGA CAAGGCCTGC AGACCGTAAA CCTGAGCCAG
       TAAAAAAGGT CTGGTCATCT GTTCCGGACG TCTGGCATTT GGACTCGGTC

12451  GCTTTCAAAA ACTTGCAGGG GCTGTGGGGG GTGCGGGCTC CCACAGGCGA
       CGAAAGTTTT TGAACGTCCC CGACACCCCC CACGCCCGAG GGTGTCCGCT

12501  CCGCGCGACC GTGTCTAGCT TGCTGACGCC CAACTCGCGC CTGTTGCTGC
       GGCGCGCTGG CACAGATCGA ACGACTGCGG GTTGAGCGCG GACAACGACG

12551  TGCTAATAGC GCCCTTCACG GACAGTGGCA GCGTGTCCCG GGACACATAC
       ACGATTATCG CGGGAAGTGC CTGTCACCGT CGCACAGGGC CCTGTGTATG

12601  CTAGGTCACT TGCTGACACT GTACCGCGAG GCCATAGGTC AGGCGCATGT
       GATCCAGTGA ACGACTGTGA CATGGCGCTC CGGTATCCAG TCCGCGTACA

12651  GGACGAGCAT ACTTTCCAGG AGATTACAAG TGTCAGCCGC GCGCTGGGGC
       CCTGCTCGTA TGAAAGGTCC TCTAATGTTC ACAGTCGGCG CGCGACCCCG
```

FIG.9A-15

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12701  AGGAGGACAC GGGCAGCCTG GAGGCAACCC TAAACTACCT GCTGACCAAC
       TCCTCCTGTG CCCGTCGGAC CTCCGTTGGG ATTTGATGGA CGACTGGTTG

12751  CGGCGGCAGA AGATCCCCTC GTTGCACAGT TTAAACAGCG AGGAGGAGCG
       GCCGCCGTCT TCTAGGGGAG CAACGTGTCA AATTTGTCGC TCCTCCTCGC

12801  CATTTTGCGC TACGTGCAGC AGAGCGTGAG CCTTAACCTG ATGCGCGACG
       GTAAAACGCG ATGCACGTCG TCTCGCACTC GGAATTGGAC TACGCGCTGC

12851  GGGTAACGCC CAGCGTGGCG CTGGACATGA CCGCGCGCAA CATGGAACCG
       CCCATTGCGG GTCGCACCGC GACCTGTACT GGCGCGCGTT GTACCTTGGC

12901  GGCATGTATG CCTCAAACCG GCCGTTTATC AACCGCCTAA TGGACTACTT
       CCGTACATAC GGAGTTTGGC CGGCAAATAG TTGGCGGATT ACCTGATGAA

12951  GCATCGCGCG GCCGCCGTGA ACCCCGAGTA TTTCACCAAT GCCATCTTGA
       CGTAGCGCGC CGGCGGCACT TGGGGCTCAT AAAGTGGTTA CGGTAGAACT

13001  ACCCGCACTG GCTACCGCCC CCTGGTTTCT ACACCGGGGG ATTCGAGGTG
       TGGGCGTGAC CGATGGCGGG GGACCAAAGA TGTGGCCCCC TAAGCTCCAC

13051  CCCGAGGGTA ACGATGGATT CCTCTGGGAC GACATAGACG ACAGCGTGTT
       GGGCTCCCAT TGCTACCTAA GGAGACCCTG CTGTATCTGC TGTCGCACAA

13101  TTCCCCGCAA CCGCAGACCC TGCTAGAGTT GCAACAGCGC GAGCAGGCAG
       AAGGGGCGTT GGCGTCTGGG ACGATCTCAA CGTTGTCGCG CTCGTCCGTC

13151  AGGCGGCGCT GCGAAAGGAA AGCTTCCGCA GGCCAAGCAG CTTGTCCGAT
       TCCGCCGCGA CGCTTTCCTT TCGAAGGCGT CCGGTTCGTC GAACAGGCTA

13201  CTAGGCGCTG CGGCCCCGCG GTCAGATGCT AGTAGCCCAT TTCCAAGCTT
       GATCCGCGAC GCCGGGGCGC CAGTCTACGA TCATCGGGTA AAGGTTCGAA

13251  GATAGGGTCT CTTACCAGCA CTCGCACCAC CCGCCCGCGC CTGCTGGGCG
       CTATCCCAGA GAATGGTCGT GAGCGTGGTG GGCGGGCGCG GACGACCCGC

13301  AGGAGGAGTA CCTAAACAAC TCGCTGCTGC AGCCGCAGCG CGAAAAAAAC
       TCCTCCTCAT GGATTTGTTG AGCGACGACG TCGGCGTCGC GCTTTTTTTG

13351  CTGCCTCCGG CATTTCCCAA CAACGGGATA GAGAGCCTAG TGGACAAGAT
       GACGGAGGCC GTAAAGGGTT GTTGCCCTAT CTCTCGGATC ACCTGTTCTA

13401  GAGTAGATGG AAGACGTACG CGCAGGAGCA CAGGGACGTG CCAGGCCCGC
       CTCATCTACC TTCTGCATGC GCGTCCTCGT GTCCCTGCAC GGTCCGGGCG

13451  GCCCGCCCAC CCGTCGTCAA AGGCACGACC GTCAGCGGGG TCTGGTGTGG
       CGGGCGGGTG GGCAGCAGTT TCCGTGCTGG CAGTCGCCCC AGACCACACC

13501  GAGGACGATG ACTCGGCAGA CGACAGCAGC GTCCTGGATT TGGGAGGGAG
       CTCCTGCTAC TGAGCCGTCT GCTGTCGTCG CAGGACCTAA ACCCTCCCTC
```

FIG.9A-16

13551 TGGCAACCCG TTTGCGCACC TTCGCCCCAG GCTGGGGAGA ATGTTTTAAA
ACCGTTGGGC AAACGCGTGG AAGCGGGGTC CGACCCCTCT TACAAAATTT

13601 AAAAAAAAAA GCATGATGCA AAATAAAAAA CTCACCAAGG CCATGGCACC
TTTTTTTTTT CGTACTACGT TTTATTTTTT GAGTGGTTCC GGTACCGTGG

13651 GAGCGTTGGT TTTCTTGTAT TCCCCTTAGT ATGCGGCGCG CGGCGATGTA
CTCGCAACCA AAAGAACATA AGGGGAATCA TACGCCGCGC GCCGCTACAT

13701 TGAGGAAGGT CCTCCTCCCT CCTACGAGAG TGTGGTGAGC GCGGCGCCAG
ACTCCTTCCA GGAGGAGGGA GGATGCTCTC ACACCACTCG CGCCGCGGTC

13751 TGGCGGCGGC GCTGGGTTCT CCCTTCGATG CTCCCCTGGA CCCGCCGTTT
ACCGCCGCCG CGACCCAAGA GGGAAGCTAC GAGGGGACCT GGGCGGCAAA

13801 GTGCCTCCGC GGTACCTGCG GCCTACCGGG GGGAGAAACA GCATCCGTTA
CACGGAGGCG CCATGGACGC CGGATGGCCC CCCTCTTTGT CGTAGGCAAT

13851 CTCTGAGTTG GCACCCCTAT TCGACACCAC CCGTGTGTAC CTGGTGGACA
GAGACTCAAC CGTGGGGATA AGCTGTGGTG GGCACACATG GACCACCTGT

13901 ACAAGTCAAC GGATGTGGCA TCCCTGAACT ACCAGAACGA CCACAGCAAC
TGTTCAGTTG CCTACACCGT AGGGACTTGA TGGTCTTGCT GGTGTCGTTG

13951 TTTCTGACCA CGGTCATTCA AAACAATGAC TACAGCCCGG GGGAGGCAAG
AAAGACTGGT GCCAGTAAGT TTTGTTACTG ATGTCGGGCC CCCTCCGTTC

14001 CACACAGACC ATCAATCTTG ACGACCGGTC GCACTGGGGC GGCGACCTGA
GTGTGTCTGG TAGTTAGAAC TGCTGGCCAG CGTGACCCCG CCGCTGGACT

14051 AAACCATCCT GCATACCAAC ATGCCAAATG TGAACGAGTT CATGTTTACC
TTTGGTAGGA CGTATGGTTG TACGGTTTAC ACTTGCTCAA GTACAAATGG

14101 AATAAGTTTA AGGCGCGGGT GATGGTGTCG CGCTTGCCTA CTAAGGACAA
TTATTCAAAT TCCGCGCCCA CTACCACAGC GCGAACGGAT GATTCCTGTT

14151 TCAGGTGGAG CTGAAATACG AGTGGGTGGA GTTCACGCTG CCCGAGGGCA
AGTCCACCTC GACTTTATGC TCACCCACCT CAAGTGCGAC GGGCTCCCGT

14201 ACTACTCCGA GACCATGACC ATAGACCTTA TGAACAACGC GATCGTGGAG
TGATGAGGCT CTGGTACTGG TATCTGGAAT ACTTGTTGCG CTAGCACCTC

14251 CACTACTTGA AAGTGGGCAG ACAGAACGGG GTTCTGGAAA GCGACATCGG
GTGATGAACT TTCACCCGTC TGTCTTGCCC CAAGACCTTT CGCTGTAGCC

14301 GGTAAAGTTT GACACCCGCA ACTTCAGACT GGGGTTTGAC CCCGTCACTG
CCATTTCAAA CTGTGGGCGT TGAAGTCTGA CCCCAAACTG GGGCAGTGAC

14351 GTCTTGTCAT GCCTGGGGTA TATACAAACG AAGCCTTCCA TCCAGACATC
CAGAACAGTA CGGACCCCAT ATATGTTTGC TTCGGAAGGT AGGTCTGTAG

FIG.9A-17

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14401  ATTTTGCTGC CAGGATGCGG GGTGGACTTC ACCCACAGCC GCCTGAGCAA
       TAAAACGACG GTCCTACGCC CCACCTGAAG TGGGTGTCGG CGGACTCGTT

14451  CTTGTTGGGC ATCCGCAAGC GGCAACCCTT CCAGGAGGGC TTTAGGATCA
       GAACAACCCG TAGGCGTTCG CCGTTGGGAA GGTCCTCCCG AAATCCTAGT

14501  CCTACGATGA TCTGGAGGGT GGTAACATTC CCGCACTGTT GGATGTGGAC
       GGATGCTACT AGACCTCCCA CCATTGTAAG GGCGTGACAA CCTACACCTG

14551  GCCTACCAGG CGAGCTTGAA AGATGACACC GAACAGGGCG GGGGTGGCGC
       CGGATGGTCC GCTCGAACTT TCTACTGTGG CTTGTCCCGC CCCCACCGCG

14601  AGGCGGCAGC AACAGCAGTG GCAGCGGCGC GGAAGAGAAC TCCAACGCGG
       TCCGCCGTCG TTGTCGTCAC CGTCGCCGCG CCTTCTCTTG AGGTTGCGCC

14651  CAGCCGCGGC AATGCAGCCG GTGGAGGACA TGAACGATCA TGCCATTCGC
       GTCGGCGCCG TTACGTCGGC CACCTCCTGT ACTTGCTAGT ACGGTAAGCG

14701  GGCGACACCT TTGCCACACG GGCTGAGGAG AAGCGCGCTG AGGCCGAAGC
       CCGCTGTGGA AACGGTGTGC CCGACTCCTC TTCGCGCGAC TCCGGCTTCG

14751  AGCGGCCGAA GCTGCCGCCC CCGCTGCGCA ACCCGAGGTC GAGAAGCCTC
       TCGCCGGCTT CGACGGCGGG GGCGACGCGT TGGGCTCCAG CTCTTCGGAG

14801  AGAAGAAACC GGTGATCAAA CCCCTGACAG AGGACAGCAA GAAACGCAGT
       TCTTCTTTGG CCACTAGTTT GGGGACTGTC TCCTGTCGTT CTTTGCGTCA

14851  TACAACCTAA TAAGCAATGA CAGCACCTTC ACCCAGTACC GCAGCTGGTA
       ATGTTGGATT ATTCGTTACT GTCGTGGAAG TGGGTCATGG CGTCGACCAT

14901  CCTTGCATAC AACTACGGCG ACCCTCAGAC CGGAATCCGC TCATGGACCC
       GGAACGTATG TTGATGCCGC TGGGAGTCTG GCCTTAGGCG AGTACCTGGG

14951  TGCTTTGCAC TCCTGACGTA ACCTGCGGCT CGGAGCAGGT CTACTGGTCG
       ACGAAACGTG AGGACTGCAT TGGACGCCGA GCCTCGTCCA GATGACCAGC

15001  TTGCCAGACA TGATGCAAGA CCCCGTGACC TTCCGCTCCA CGCGCCAGAT
       AACGGTCTGT ACTACGTTCT GGGGCACTGG AAGGCGAGGT GCGCGGTCTA

15051  CAGCAACTTT CCGGTGGTGG GCGCCGAGCT GTTGCCCGTG CACTCCAAGA
       GTCGTTGAAA GGCCACCACC CGCGGCTCGA CAACGGGCAC GTGAGGTTCT

15101  GCTTCTACAA CGACCAGGCC GTCTACTCCC AACTCATCCG CCAGTTTACC
       CGAAGATGTT GCTGGTCCGG CAGATGAGGG TTGAGTAGGC GGTCAAATGG

15151  TCTCTGACCC ACGTGTTCAA TCGCTTTCCC GAGAACCAGA TTTTGGCGCG
       AGAGACTGGG TGCACAAGTT AGCGAAAGGG CTCTTGGTCT AAAACCGCGC

15201  CCCGCCAGCC CCCACCATCA CCACCGTCAG TGAAAACGTT CCTGCTCTCA
       GGGCGGTCGG GGGTGGTAGT GGTGGCAGTC ACTTTTGCAA GGACGAGAGT
```

FIG.9A-18

15251 CAGATCACGG GACGCTACCG CTGCGCAACA GCATCGGAGG AGTCCAGCGA
GTCTAGTGCC CTGCGATGGC GACGCGTTGT CGTAGCCTCC TCAGGTCGCT

15301 GTGACCATTA CTGACGCCAG ACGCCGCACC TGCCCCTACG TTTACAAGGC
CACTGGTAAT GACTGCGGTC TGCGGCGTGG ACGGGGATGC AAATGTTCCG

15351 CCTGGGCATA GTCTCGCCGC GCGTCCTATC GAGCCGCACT TTTTGAGCAA
GGACCCGTAT CAGAGCGGCG CGCAGGATAG CTCGGCGTGA AAAACTCGTT

15401 GCATGTCCAT CCTTATATCG CCCAGCAATA ACACAGGCTG GGGCCTGCGC
CGTACAGGTA GGAATATAGC GGGTCGTTAT TGTGTCCGAC CCCGGACGCG

15451 TTCCCAAGCA AGATGTTTGG CGGGGCCAAG AAGCGCTCCG ACCAACACCC
AAGGGTTCGT TCTACAAACC GCCCCGGTTC TTCGCGAGGC TGGTTGTGGG

15501 AGTGCGCGTG CGCGGGCACT ACCGCGCGCC CTGGGGCGCG CACAAACGCG
TCACGCGCAC GCGCCCGTGA TGGCGCGCGG GACCCCGCGC GTGTTTGCGC

15551 GCCGCACTGG GCGCACCACC GTCGATGACG CCATCGACGC GGTGGTGGAG
CGGCGTGACC CGCGTGGTGG CAGCTACTGC GGTAGCTGCG CCACCACCTC

15601 GAGGCGCGCA ACTACACGCC CACGCCGCCA CCAGTGTCCA CAGTGGACGC
CTCCGCGCGT TGATGTGCGG GTGCGGCGGT GGTCACAGGT GTCACCTGCG

15651 GGCCATTCAG ACCGTGGTGC GCGGAGCCCG GCGCTATGCT AAAATGAAGA
CCGGTAAGTC TGGCACCACG CGCCTCGGGC CGCGATACGA TTTTACTTCT

15701 GACGGCGGAG GCGCGTAGCA CGTCGCCACC GCCGCCGACC CGGCACTGCC
CTGCCGCCTC CGCGCATCGT GCAGCGGTGG CGGCGGCTGG GCCGTGACGG

15751 GCCCAACGCG CGGCGGCGGC CCTGCTTAAC CGCGCACGTC GCACCGGCCG
CGGGTTGCGC GCCGCCGCCG GGACGAATTG GCGCGTGCAG CGTGGCCGGC

15801 ACGGGCGGCC ATGCGGGCCG CTCGAAGGCT GGCCGCGGGT ATTGTCACTG
TGCCCGCCGG TACGCCCGGC GAGCTTCCGA CCGGCGCCCA TAACAGTGAC

15851 TGCCCCCCAG GTCCAGGCGA CGAGCGGCCG CCGCAGCAGC CGCGGCCATT
ACGGGGGGTC CAGGTCCGCT GCTCGCCGGC GGCGTCGTCG GCGCCGGTAA

15901 AGTGCTATGA CTCAGGGTCG CAGGGGCAAC GTGTATTGGG TGCGCGACTC
TCACGATACT GAGTCCCAGC GTCCCCGTTG CACATAACCC ACGCGCTGAG

15951 GGTTAGCGGC CTGCGCGTGC CCGTGCGCAC CCGCCCCCCG CGCAACTAGA
CCAATCGCCG GACGCGCACG GGCACGCGTG GGCGGGGGGC GCGTTGATCT

16001 TTGCAAGAAA AAACTACTTA GACTCGTACT GTTGTATGTA TCCAGCGGCG
AACGTTCTTT TTTGATGAAT CTGAGCATGA CAACATACAT AGGTCGCCGC

16051 GCGGCGCGCA ACGAAGCTAT GTCCAAGCGC AAAATCAAAG AAGAGATGCT
CGCCGCGCGT TGCTTCGATA CAGGTTCGCG TTTTAGTTTC TTCTCTACGA

FIG.9A-19

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16101  CCAGGTCATC GCGCCGGAGA TCTATGGCCC CCCGAAGAAG GAAGAGCAGG
       GGTCCAGTAG CGCGGCCTCT AGATACCGGG GGGCTTCTTC CTTCTCGTCC

16151  ATTACAAGCC CCGAAAGCTA AAGCGGGTCA AAAAGAAAAA GAAAGATGAT
       TAATGTTCGG GGCTTTCGAT TTCGCCCAGT TTTTCTTTTT CTTTCTACTA

16201  GATGATGAAC TTGACGACGA GGTGGAACTG CTGCACGCTA CCGCGCCCAG
       CTACTACTTG AACTGCTGCT CCACCTTGAC GACGTGCGAT GGCGCGGGTC

16251  GCGACGGGTA CAGTGGAAAG GTCGACGCGT AAAACGTGTT TTGCGACCCG
       CGCTGCCCAT GTCACCTTTC CAGCTGCGCA TTTTGCACAA AACGCTGGGC

16301  GCACCACCGT AGTCTTTTACG CCCGGTGAGC GCTCCACCCG CACCTACAAG
       CGTGGTGGCA TCAGAAATGC GGGCCACTCG CGAGGTGGGC GTGGATGTTC

16351  CGCGTGTATG ATGAGGTGTA CGGCGACGAG GACCTGCTTG AGCAGGCCAA
       GCGCACATAC TACTCCACAT GCCGCTGCTC CTGGACGAAC TCGTCCGGTT

16401  CGAGCGCCTC GGGGAGTTTG CCTACGGAAA GCGGCATAAG GACATGCTGG
       GCTCGCGGAG CCCCTCAAAC GGATGCCTTT CGCCGTATTC CTGTACGACC

16451  CGTTGCCGCT GGACGAGGGC AACCCAACAC CTAGCCTAAA GCCCGTAACA
       GCAACGGCGA CCTGCTCCCG TTGGGTTGTG GATCGGATTT CGGGCATTGT

16501  CTGCAGCAGG TGCTGCCCGC GCTTGCACCG TCCGAAGAAA AGCGCGGCCT
       GACGTCGTCC ACGACGGGCG CGAACGTGGC AGGCTTCTTT TCGCGCCGGA

16551  AAAGCGCGAG TCTGGTGACT TGGCACCCAC CGTGCAGCTG ATGGTACCCA
       TTTCGCGCTC AGACCACTGA ACCGTGGGTG GCACGTCGAC TACCATGGGT

16601  AGCGCCAGCG ACTGGAAGAT GTCTTGGAAA AAATGACCGT GGAACCTGGG
       TCGCGGTCGC TGACCTTCTA CAGAACCTTT TTTACTGGCA CCTTGGACCC

16651  CTGGAGCCCG AGGTCCGCGT GCGGCCAATC AAGCAGGTGG CGCCGGGACT
       GACCTCGGGC TCCAGGCGCA CGCCGGTTAG TTCGTCCACC GCGGCCCTGA

16701  GGGCGTGCAG ACCGTGGACG TTCAGATACC CACTACCAGT AGCACCAGTA
       CCCGCACGTC TGGCACCTGC AAGTCTATGG GTGATGGTCA TCGTGGTCAT

16751  TTGCCACCGC CACAGAGGGC ATGGAGACAC AAACGTCCCC GGTTGCCTCA
       AACGGTGGCG GTGTCTCCCG TACCTCTGTG TTTGCAGGGG CCAACGGAGT

16801  GCGGTGGCGG ATGCCGCGGT GCAGGCGGTC GCTGCGGCCG CGTCCAAGAC
       CGCCACCGCC TACGGCGCCA CGTCCGCCAG CGACGCCGGC GCAGGTTCTG

16851  CTCTACGGAG GTGCAAACGG ACCCGTGGAT GTTTCGCGTT TCAGCCCCCC
       GAGATGCCTC CACGTTTGCC TGGGCACCTA CAAAGCGCAA AGTCGGGGGG

16901  GGCGCCCGCG CCGTTCGAGG AAGTACGGCG CCGCCAGCGC GCTACTGCCC
       CCGCGGGCGC GGCAAGCTCC TTCATGCCGC GGCGGTCGCG CGATGACGGG
```

FIG.9A-20

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16951  GAATATGCCC TACATCCTTC CATTGCGCCT ACCCCCGGCT ATCGTGGCTA
       CTTATACGGG ATGTAGGAAG GTAACGCGGA TGGGGGCCGA TAGCACCGAT

17001  CACCTACCGC CCCAGAAGAC GAGCAACTAC CCGACGCCGA ACCACCACTG
       GTGGATGGCG GGGTCTTCTG CTCGTTGATG GGCTGCGGCT TGGTGGTGAC

17051  GAACCCGCCG CCGCCGTCGC CGTCGCCAGC CCGTGCTGGC CCCGATTTCC
       CTTGGGCGGC GGCGGCAGCG GCAGCGGTCG GGCACGACCG GGGCTAAAGG

17101  GTGCGCAGGG TGGCTCGCGA AGGAGGCAGG ACCCTGGTGC TGCCAACAGC
       CACGCGTCCC ACCGAGCGCT TCCTCCGTCC TGGGACCACG ACGGTTGTCG

17151  GCGCTACCAC CCCAGCATCG TTTAAAAGCC GGTCTTTGTG GTTCTTGCAG
       CGCGATGGTG GGGTCGTAGC AAATTTTCGG CCAGAAACAC CAAGAACGTC

17201  ATATGGCCCT CACCTGCCGC CTCCGTTTCC CGGTGCCGGG ATTCCGAGGA
       TATACCGGGA GTGGACGGCG GAGGCAAAGG GCCACGGCCC TAAGGCTCCT

17251  AGAATGCACC GTAGGAGGGG CATGGCCGGC CACGGCCTGA CGGGCGGCAT
       TCTTACGTGG CATCCTCCCC GTACCGGCCG GTGCCGGACT GCCCGCCGTA

17301  GCGTCGTGCG CACCACCGGC GGCGGCGCGC GTCGCACCGT CGCATGCGCG
       CGCAGCACGC GTGGTGGCCG CCGCCGCGCG CAGCGTGGCA GCGTACGCGC

17351  GCGGTATCCT GCCCCTCCTT ATTCCACTGA TCGCCGCGGC GATTGGCGCC
       CGCCATAGGA CGGGGAGGAA TAAGGTGACT AGCGGCGCCG CTAACCGCGG

17401  GTGCCCGGAA TTGCATCCGT GGCCTTGCAG GCGCAGAGAC ACTGATTAAA
       CACGGGCCTT AACGTAGGCA CCGGAACGTC CGCGTCTCTG TGACTAATTT

17451  AACAAGTTGC ATGTGGAAAA ATCAAAATAA AAAGTCTGGA CTCTCACGCT
       TTGTTCAACG TACACCTTTT TAGTTTTATT TTTCAGACCT GAGAGTGCGA

17501  CGCTTGGTCC TGTAACTATT TTGTAGAATG GAAGACATCA ACTTTGCGTC
       GCGAACCAGG ACATTGATAA AACATCTTAC CTTCTGTAGT TGAAACGCAG

17551  TCTGGCCCCG CGACACGGCT CGCGCCCGTT CATGGGAAAC TGGCAAGATA
       AGACCGGGGC GCTGTGCCGA GCGCGGGCAA GTACCCTTTG ACCGTTCTAT

17601  TCGGCACCAG CAATATGAGC GGTGGCGCCT TCAGCTGGGG CTCGCTGTGG
       AGCCGTGGTC GTTATACTCG CCACCGCGGA AGTCGACCCC GAGCGACACC

17651  AGCGGCATTA AAAATTTCGG TTCCACCGTT AAGAACTATG GCAGCAAGGC
       TCGCCGTAAT TTTTAAAGCC AAGGTGGCAA TTCTTGATAC CGTCGTTCCG

17701  CTGGAACAGC AGCACAGGCC AGATGCTGAG GGATAAGTTG AAAGAGCAAA
       GACCTTGTCG TCGTGTCCGG TCTACGACTC CCTATTCAAC TTTCTCGTTT

17751  ATTTCCAACA AAAGGTGGTA GATGGCCTGG CCTCTGGCAT TAGCGGGGTG
       TAAAGGTTGT TTTCCACCAT CTACCGGACC GGAGACCGTA ATCGCCCCAC
```

FIG.9A-21

17801 GTGGACCTGG CCAACCAGGC AGTGCAAAAT AAGATTAACA GTAAGCTTGA
CACCTGGACC GGTTGGTCCG TCACGTTTTA TTCTAATTGT CATTCGAACT

17851 TCCCCGCCCT CCCGTAGAGG AGCCTCCACC GGCCGTGGAG ACAGTGTCTC
AGGGGCGGGA GGGCATCTCC TCGGAGGTGG CCGGCACCTC TGTCACAGAG

17901 CAGAGGGGCG TGGCGAAAAG CGTCCGCGCC CCGACAGGGA AGAAACTCTG
GTCTCCCCGC ACCGCTTTTC GCAGGCGCGG GGCTGTCCCT TCTTTGAGAC

17951 GTGACGCAAA TAGACGAGCC TCCCTCGTAC GAGGAGGCAC TAAAGCAAGG
CACTGCGTTT ATCTGCTCGG AGGGAGCATG CTCCTCCGTG ATTTCGTTCC

18001 CCTGCCCACC ACCCGTCCCA TCGCGCCCAT GGCTACCGGA GTGCTGGGCC
GGACGGGTGG TGGGCAGGGT AGCGCGGGTA CCGATGGCCT CACGACCCGG

18051 AGCACACACC CGTAACGCTG GACCTGCCTC CCCCGCCGA CACCCAGCAG
TCGTGTGTGG GCATTGCGAC CTGGACGGAG GGGGGCGGCT GTGGGTCGTC

18101 AAACCTGTGC TGCCAGGCCC GACCGCCGTT GTTGTAACCC GTCCTAGCCG
TTTGGACACG ACGGTCCGGG CTGGCGGCAA CAACATTGGG CAGGATCGGC

18151 CGCGTCCCTG CGCCGCGCCG CCAGCGGTCC GCGATCGTTG CGGCCCGTAG
GCGCAGGGAC GCGGCGCGGC GGTCGCCAGG CGCTAGCAAC GCCGGGCATC

18201 CCAGTGGCAA CTGGCAAAGC ACACTGAACA GCATCGTGGG TCTGGGGGTG
GGTCACCGTT GACCGTTTCG TGTGACTTGT CGTAGCACCC AGACCCCCAC

18251 CAATCCCTGA AGCGCCGACG ATGCTTCTGA TAGCTAACGT GTCGTATGTG
GTTAGGGACT TCGCGGCTGC TACGAAGACT ATCGATTGCA CAGCATACAC

18301 TGTCATGTAT GCGTCCATGT CGCCGCCAGA GGAGCTGCTG AGCCGCCGCG
ACAGTACATA CGCAGGTACA GCGGCGGTCT CCTCGACGAC TCGGCGGCGC

18351 CGCCCGCTTT CCAAGATGGC TACCCCTTCG ATGATGCCGC AGTGGTCTTA
GCGGGCGAAA GGTTCTACCG ATGGGGAAGC TACTACGGCG TCACCAGAAT

18401 CATGCACATC TCGGGCCAGG ACGCCTCGGA GTACCTGAGC CCCGGGCTGG
GTACGTGTAG AGCCCGGTCC TGCGGAGCCT CATGGACTCG GGGCCCGACC

18451 TGCAGTTTGC CCGCGCCACC GAGACGTACT TCAGCCTGAA TAACAAGTTT
ACGTCAAACG GGCGCGGTGG CTCTGCATGA AGTCGGACTT ATTGTTCAAA

18501 AGAAACCCCA CGGTGGCGCC TACGCACGAC GTGACCACAG ACCGGTCCCA
TCTTTGGGGT GCCACCGCGG ATGCGTGCTG CACTGGTGTC TGGCCAGGGT

18551 GCGTTTGACG CTGCGGTTCA TCCCTGTGGA CCGTGAGGAT ACTGCGTACT
CGCAAACTGC GACGCCAAGT AGGGACACCT GGCACTCCTA TGACGCATGA

18601 CGTACAAGGC GCGGTTCACC CTAGCTGTGG GTGATAACCG TGTGCTGGAC
GCATGTTCCG CGCCAAGTGG GATCGACACC CACTATTGGC ACACGACCTG

FIG.9A-22

18651 ATGGCTTCCA CGTACTTTGA CATCCGCGGC GTGCTGGACA GGGGCCCTAC
TACCGAAGGT GCATGAAACT GTAGGCGCCG CACGACCTGT CCCCGGGATG

18701 TTTTAAGCCC TACTCTGGCA CTGCCTACAA CGCCCTGGCT CCCAAGGGTG
AAAATTCGGG ATGAGACCGT GACGGATGTT GCGGGACCGA GGGTTCCCAC

18751 CCCCAAATCC TTGCGAATGG GATGAAGCTG CTACTGCTCT TGAAATAAAC
GGGGTTTAGG AACGCTTACC CTACTTCGAC GATGACGAGA ACTTTATTTG

18801 CTAGAAGAAG AGGACGATGA CAACGAAGAC GAAGTAGACG AGCAAGCTGA
GATCTTCTTC TCCTGCTACT GTTGCTTCTG CTTCATCTGC TCGTTCGACT

18851 GCAGCAAAAA ACTCACGTAT TTGGGCAGGC GCCTTATTCT GGTATAAATA
CGTCGTTTTT TGAGTGCATA AACCCGTCCG CGGAATAAGA CCATATTTAT

18901 TTACAAAGGA GGGTATTCAA ATAGGTGTCG AAGGTCAAAC ACCTAAATAT
AATGTTTCCT CCCATAAGTT TATCCACAGC TTCCAGTTTG TGGATTTATA

18951 GCCGATAAAA CATTTCAACC TGAACCTCAA ATAGGAGAAT CTCAGTGGTA
CGGCTATTTT GTAAAGTTGG ACTTGGAGTT TATCCTCTTA GAGTCACCAT

19001 CGAAACAGAA ATTAATCATG CAGCTGGGAG AGTCCTAAAA AAGACTACCC
GCTTTGTCTT TAATTAGTAC GTCGACCCTC TCAGGATTTT TTCTGATGGG

19051 CAATGAAACC ATGTTACGGT TCATATGCAA AACCCACAAA TGAAAATGGA
GTTACTTTGG TACAATGCCA AGTATACGTT TTGGGTGTTT ACTTTTACCT

19101 GGGCAAGGCA TTCTTGTAAA GCAACAAAAT GGAAAGCTAG AAAGTCAAGT
CCCGTTCCGT AAGAACATTT CGTTGTTTTA CCTTTCGATC TTTCAGTTCA

19151 GGAAATGCAA TTTTTCTCAA CTACTGAGGC AGCCGCAGGC AATGGTGATA
CCTTTACGTT AAAAAGAGTT GATGACTCCG TCGGCGTCCG TTACCACTAT

19201 ACTTGACTCC TAAAGTGGTA TTGTACAGTG AAGATGTAGA TATAGAAACC
TGAACTGAGG ATTTCACCAT AACATGTCAC TTCTACATCT ATATCTTTGG

19251 CCAGACACTC ATATTTCTTA CATGCCCACT ATTAAGGAAG GTAACTCACG
GGTCTGTGAG TATAAAGAAT GTACGGGTGA TAATTCCTTC CATTGAGTGC

19301 AGAACTAATG GGCCAACAAT CTATGCCCAA CAGGCCTAAT TACATTGCTT
TCTTGATTAC CCGGTTGTTA GATACGGGTT GTCCGGATTA ATGTAACGAA

19351 TTAGGGACAA TTTTATTGGT CTAATGTATT ACAACAGCAC GGGTAATATG
AATCCCTGTT AAAATAACCA GATTACATAA TGTTGTCGTG CCCATTATAC

19401 GGTGTTCTGG CGGGCCAAGC ATCGCAGTTG AATGCTGTTG TAGATTTGCA
CCACAAGACC GCCCGGTTCG TAGCGTCAAC TTACGACAAC ATCTAAACGT

19451 AGACAGAAAC ACAGAGCTTT CATACCAGCT TTTGCTTGAT TCCATTGGTG
TCTGTCTTTG TGTCTCGAAA GTATGGTCGA AAACGAACTA AGGTAACCAC

FIG.9A-23

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19501  ATAGAACCAG GTACTTTTCT ATGTGGAATC AGGCTGTTGA CAGCTATGAT
       TATCTTGGTC CATGAAAAGA TACACCTTAG TCCGACAACT GTCGATACTA

19551  CCAGATGTTA GAATTATTGA AAATCATGGA ACTGAAGATG AACTTCCAAA
       GGTCTACAAT CTTAATAACT TTTAGTACCT TGACTTCTAC TTGAAGGTTT

19601  TTACTGCTTT CCACTGGGAG GTGTGATTAA TACAGAGACT CTTACCAAGG
       AATGACGAAA GGTGACCCTC CACACTAATT ATGTCTCTGA GAATGGTTCC

19651  TAAAACCTAA AACAGGTCAG GAAAATGGAT GGGAAAAAGA TGCTACAGAA
       ATTTTGGATT TTGTCCAGTC CTTTTACCTA CCCTTTTTCT ACGATGTCTT

19701  TTTTCAGATA AAAATGAAAT AAGAGTTGGA AATAATTTTG CCATGGAAAT
       AAAAGTCTAT TTTTACTTTA TTCTCAACCT TTATTAAAAC GGTACCTTTA

19751  CAATCTAAAT GCCAACCTGT GGAGAAATTT CCTGTACTCC AACATAGCGC
       GTTAGATTTA CGGTTGGACA CCTCTTTAAA GGACATGAGG TTGTATCGCG

19801  TGTATTTGCC CGACAAGCTA AAGTACAGTC CTTCCAACGT AAAAATTTCT
       ACATAAACGG GCTGTTCGAT TTCATGTCAG GAAGGTTGCA TTTTTAAAGA

19851  GATAACCCAA ACACCTACGA CTACATGAAC AAGCGAGTGG TGGCTCCCGG
       CTATTGGGTT TGTGGATGCT GATGTACTTG TTCGCTCACC ACCGAGGGCC

19901  GCTAGTGGAC TGCTACATTA ACCTTGGAGC ACGCTGGTCC CTTGACTATA
       CGATCACCTG ACGATGTAAT TGGAACCTCG TGCGACCAGG GAACTGATAT

19951  TGGACAACGT CAACCCATTT AACCACCACC GCAATGCTGG CCTGCGCTAC
       ACCTGTTGCA GTTGGGTAAA TTGGTGGTGG CGTTACGACC GGACGCGATG

20001  CGCTCAATGT TGCTGGGCAA TGGTCGCTAT GTGCCCTTCC ACATCCAGGT
       GCGAGTTACA ACGACCCGTT ACCAGCGATA CACGGGAAGG TGTAGGTCCA

20051  GCCTCAGAAG TTCTTTGCCA TTAAAAACCT CCTTCTCCTG CCGGGCTCAT
       CGGAGTCTTC AAGAAACGGT AATTTTTGGA GGAAGAGGAC GGCCCGAGTA

20101  ACACCTACGA GTGGAACTTC AGGAAGGATG TTAACATGGT TCTGCAGAGC
       TGTGGATGCT CACCTTGAAG TCCTTCCTAC AATTGTACCA AGACGTCTCG

20151  TCCCTAGGAA ATGACCTAAG GGTTGACGGA GCCAGCATTA AGTTTGATAG
       AGGGATCCTT TACTGGATTC CCAACTGCCT CGGTCGTAAT TCAAACTATC

20201  CATTTGCCTT TACGCCACCT TCTTCCCCAT GGCCCACAAC ACCGCCTCCA
       GTAAACGGAA ATGCGGTGGA AGAAGGGGTA CCGGGTGTTG TGGCGGAGGT

20251  CGCTTGAGGC CATGCTTAGA AACGACACCA ACGACCAGTC CTTTAACGAC
       GCGAACTCCG GTACGAATCT TTGCTGTGGT TGCTGGTCAG GAAATTGCTG

20301  TATCTCTCCG CCGCCAACAT GCTCTACCCT ATACCCGCCA ACGCTACCAA
       ATAGAGAGGC GGCGGTTGTA CGAGATGGGA TATGGGCGGT TGCGATGGTT
```

FIG.9A-24

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20351  CGTGCCCATA TCCATCCCCT CCCGCAACTG GGCGGCTTTC CGCGGCTGGG
       GCACGGGTAT AGGTAGGGGA GGGCGTTGAC CCGCCGAAAG GCGCCGACCC

20401  CCTTCACGCG CCTTAAGACT AAGGAAACCC CATCACTGGG CTCGGGCTAC
       GGAAGTGCGC GGAATTCTGA TTCCTTTGGG GTAGTGACCC GAGCCCGATG

20451  GACCCTTATT ACACCTACTC TGGCTCTATA CCCTACCTAG ATGGAACCTT
       CTGGGAATAA TGTGGATGAG ACCGAGATAT GGGATGGATC TACCTTGGAA

20501  TTACCTCAAC CACACCTTTA AGAAGGTGGC CATTACCTTT GACTCTTCTG
       AATGGAGTTG GTGTGGAAAT TCTTCCACCG GTAATGGAAA CTGAGAAGAC

20551  TCAGCTGGCC TGGCAATGAC CGCCTGCTTA CCCCCAACGA GTTTGAAATT
       AGTCGACCGG ACCGTTACTG GCGGACGAAT GGGGGTTGCT CAAACTTTAA

20601  AAGCGCTCAG TTGACGGGGA GGGTTACAAC GTTGCCCAGT GTAACATGAC
       TTCGCGAGTC AACTGCCCCT CCCAATGTTG CAACGGGTCA CATTGTACTG

20651  CAAAGACTGG TTCCTGGTAC AAATGCTAGC TAACTATAAC ATTGGCTACC
       GTTTCTGACC AAGGACCATG TTTACGATCG ATTGATATTG TAACCGATGG

20701  AGGGCTTCTA TATCCCAGAG AGCTACAAGG ACCGCATGTA CTCCTTCTTT
       TCCCGAAGAT ATAGGGTCTC TCGATGTTCC TGGCGTACAT GAGGAAGAAA

20751  AGAAACTTCC AGCCCATGAG CCGTCAGGTG GTGGATGATA CTAAATACAA
       TCTTTGAAGG TCGGGTACTC GGCAGTCCAC CACCTACTAT GATTTATGTT

20801  GGACTACCAA CAGGTGGGCA TCCTACACCA ACACAACAAC TCTGGATTTG
       CCTGATGGTT GTCCACCCGT AGGATGTGGT TGTGTTGTTG AGACCTAAAC

20851  TTGGCTACCT TGCCCCCACC ATGCGCGAAG GACAGGCCTA CCCTGCTAAC
       AACCGATGGA ACGGGGGTGG TACGCGCTTC CTGTCCGGAT GGGACGATTG

20901  TTCCCCTATC CGCTTATAGG CAAGACCGCA GTTGACAGCA TTACCCAGAA
       AAGGGGATAG GCGAATATCC GTTCTGGCGT CAACTGTCGT AATGGGTCTT

20951  AAAGTTTCTT TGCGATCGCA CCCTTTGGCG CATCCCATTC TCCAGTAACT
       TTTCAAAGAA ACGCTAGCGT GGGAAACCGC GTAGGGTAAG AGGTCATTGA

21001  TTATGTCCAT GGGCGCACTC ACAGACCTGG GCCAAAACCT TCTCTACGCC
       AATACAGGTA CCCGCGTGAG TGTCTGGACC CGGTTTTGGA AGAGATGCGG

21051  AACTCCGCCC ACGCGCTAGA CATGACTTTT GAGGTGGATC CCATGGACGA
       TTGAGGCGGG TGCGCGATCT GTACTGAAAA CTCCACCTAG GGTACCTGCT

21101  GCCCACCCTT CTTTATGTTT TGTTTGAAGT CTTTGACGTG GTCCGTGTGC
       CGGGTGGGAA GAAATACAAA ACAAACTTCA GAAACTGCAC CAGGCACACG

21151  ACCAGCCGCA CCGCGGCGTC ATCGAAACCG TGTACCTGCG CACGCCCTTC
       TGGTCGGCGT GGCGCCGCAG TAGCTTTGGC ACATGGACGC GTGCGGGAAG
```

FIG.9A-25

```
21201  TCGGCCGGCA ACGCCACAAC ATAAAGAAGC AAGCAACATC AACAACAGCT
       AGCCGGCCGT TGCGGTGTTG TATTTCTTCG TTCGTTGTAG TTGTTGTCGA

21251  GCCGCCATGG GCTCCAGTGA GCAGGAACTG AAAGCCATTG TCAAAGATCT
       CGGCGGTACC CGAGGTCACT CGTCCTTGAC TTTCGGTAAC AGTTTCTAGA

21301  TGGTTGTGGG CCATATTTTT TGGGCACCTA TGACAAGCGC TTTCCAGGCT
       ACCAACACCC GGTATAAAAA ACCCGTGGAT ACTGTTCGCG AAAGGTCCGA

21351  TTGTTTCTCC ACACAAGCTC GCCTGCGCCA TAGTCAATAC GGCCGGTCGC
       AACAAAGAGG TGTGTTCGAG CGGACGCGGT ATCAGTTATG CCGGCCAGCG

21401  GAGACTGGGG GCGTACACTG GATGGCCTTT GCCTGGAACC CGCACTCAAA
       CTCTGACCCC CGCATGTGAC CTACCGGAAA CGGACCTTGG GCGTGAGTTT

21451  AACATGCTAC CTCTTTGAGC CCTTTGGCTT TTCTGACCAG CGACTCAAGC
       TTGTACGATG GAGAAACTCG GGAAACCGAA AAGACTGGTC GCTGAGTTCG

21501  AGGTTTACCA GTTTGAGTAC GAGTCACTCC TGCGCCGTAG CGCCATTGCT
       TCCAAATGGT CAAACTCATG CTCAGTGAGG ACGCGGCATC GCGGTAACGA

21551  TCTTCCCCCG ACCGCTGTAT AACGCTGGAA AAGTCCACCC AAAGCGTACA
       AGAAGGGGGC TGGCGACATA TTGCGACCTT TTCAGGTGGG TTTCGCATGT

21601  GGGGCCCAAC TCGGCCGCCT GTGGACTATT CTGCTGCATG TTTCTCCACG
       CCCCGGGTTG AGCCGGCGGA CACCTGATAA GACGACGTAC AAAGAGGTGC

21651  CCTTTGCCAA CTGGCCCCAA ACTCCCATGG ATCACAACCC CACCATGAAC
       GGAAACGGTT GACCGGGGTT TGAGGGTACC TAGTGTTGGG GTGGTACTTG

21701  CTTATTACCG GGGTACCCAA CTCCATGCTC AACAGTCCCC AGGTACAGCC
       GAATAATGGC CCCATGGGTT GAGGTACGAG TTGTCAGGGG TCCATGTCGG

21751  CACCCTGCGT CGCAACCAGG AACAGCTCTA CAGCTTCCTG GAGCGCCACT
       GTGGGACGCA GCGTTGGTCC TTGTCGAGAT GTCGAAGGAC CTCGCGGTGA

21801  CGCCCTACTT CCGCAGCCAC AGTGCGCAGA TTAGGAGCGC CACTTCTTTT
       GCGGGATGAA GGCGTCGGTG TCACGCGTCT AATCCTCGCG GTGAAGAAAA

21851  TGTCACTTGA AAAACATGTA AAAATAATGT ACTAGAGACA CTTTCAATAA
       ACAGTGAACT TTTTGTACAT TTTTATTACA TGATCTCTGT GAAAGTTATT

21901  AGGCAAATGC TTTTATTTGT ACACTCTCGG GTGATTATTT ACCCCCACCC
       TCCGTTTACG AAAATAAACA TGTGAGAGCC CACTAATAAA TGGGGGTGGG

21951  TTGCCGTCTG CGCCGTTTAA AAATCAAAGG GGTTCTGCCG CGCATCGCTA
       AACGGCAGAC GCGGCAAATT TTTAGTTTCC CCAAGACGGC GCGTAGCGAT

22001  TGCGCCACTG GCAGGGACAC GTTGCGATAC TGGTGTTTAG TGCTCCACTT
       ACGCGGTGAC CGTCCCTGTG CAACGCTATG ACCACAAATC ACGAGGTGAA
```

FIG.9A-26

```
22051  AAACTCAGGC ACAACCATCC GCGGCAGCTC GGTGAAGTTT TCACTCCACA
       TTTGAGTCCG TGTTGGTAGG CGCCGTCGAG CCACTTCAAA AGTGAGGTGT

22101  GGCTGCGCAC CATCACCAAC GCGTTTAGCA GGTCGGGCGC CGATATCTTG
       CCGACGCGTG GTAGTGGTTG CGCAAATCGT CCAGCCCGCG GCTATAGAAC

22151  AAGTCGCAGT TGGGGCCTCC GCCCTGCGCG CGCGAGTTGC GATACACAGG
       TTCAGCGTCA ACCCCGGAGG CGGGACGCGC GCGCTCAACG CTATGTGTCC

22201  GTTGCAGCAC TGGAACACTA TCAGCGCCGG GTGGTGCACG CTGGCCAGCA
       CAACGTCGTG ACCTTGTGAT AGTCGCGGCC CACCACGTGC GACCGGTCGT

22251  CGCTCTTGTC GGAGATCAGA TCCGCGTCCA GGTCCTCCGC GTTGCTCAGG
       GCGAGAACAG CCTCTAGTCT AGGCGCAGGT CCAGGAGGCG CAACGAGTCC

22301  GCGAACGGAG TCAACTTTGG TAGCTGCCTT CCCAAAAAGG GCGCGTGCCC
       CGCTTGCCTC AGTTGAAACC ATCGACGGAA GGGTTTTTCC CGCGCACGGG

22351  AGGCTTTGAG TTGCACTCGC ACCGTAGTGG CATCAAAAGG TGACCGTGCC
       TCCGAAACTC AACGTGAGCG TGGCATCACC GTAGTTTTCC ACTGGCACGG

22401  CGGTCTGGGC GTTAGGATAC AGCGCCTGCA TAAAAGCCTT GATCTGCTTA
       GCCAGACCCG CAATCCTATG TCGCGGACGT ATTTTCGGAA CTAGACGAAT

22451  AAAGCCACCT GAGCCTTTGC GCCTTCAGAG AAGAACATGC CGCAAGACTT
       TTTCGGTGGA CTCGGAAACG CGGAAGTCTC TTCTTGTACG GCGTTCTGAA

22501  GCCGGAAAAC TGATTGGCCG GACAGGCCGC GTCGTGCACG CAGCACCTTG
       CGGCCTTTTG ACTAACCGGC CTGTCCGGCG CAGCACGTGC GTCGTGGAAC

22551  CGTCGGTGTT GGAGATCTGC ACCACATTTC GGCCCCACCG GTTCTTCACG
       GCAGCCACAA CCTCTAGACG TGGTGTAAAG CCGGGGTGGC CAAGAAGTGC

22601  ATCTTGGCCT TGCTAGACTG CTCCTTCAGC GCGCGCTGCC CGTTTTCGCT
       TAGAACCGGA ACGATCTGAC GAGGAAGTCG CGCGCGACGG GCAAAAGCGA

22651  CGTCACATCC ATTTCAATCA CGTGCTCCTT ATTTATCATA ATGCTTCCGT
       GCAGTGTAGG TAAAGTTAGT GCACGAGGAA TAAATAGTAT TACGAAGGCA

22701  GTAGACACTT AAGCTCGCCT TCGATCTCAG CGCAGCGGTG CAGCCACAAC
       CATCTGTGAA TTCGAGCGGA AGCTAGAGTC GCGTCGCCAC GTCGGTGTTG

22751  GCGCAGCCCG TGGGCTCGTG ATGCTTGTAG GTCACCTCTG CAAACGACTG
       CGCGTCGGGC ACCCGAGCAC TACGAACATC CAGTGGAGAC GTTTGCTGAC

22801  CAGGTACGCC TGCAGGAATC GCCCCATCAT CGTCACAAAG GTCTTGTTGC
       GTCCATGCGG ACGTCCTTAG CGGGGTAGTA GCAGTGTTTC CAGAACAACG

22851  TGGTGAAGGT CAGCTGCAAC CCGCGGTGCT CCTCGTTCAG CCAGGTCTTG
       ACCACTTCCA GTCGACGTTG GGCGCCACGA GGAGCAAGTC GGTCCAGAAC
```

FIG.9A-27

```
22901  CATACGGCCG CCAGAGCTTC CACTTGGTCA GGCAGTAGTT TGAAGTTCGC
       GTATGCCGGC GGTCTCGAAG GTGAACCAGT CCGTCATCAA ACTTCAAGCG

22951  CTTTAGATCG TTATCCACGT GGTACTTGTC CATCAGCGCG CGCGCAGCCT
       GAAATCTAGC AATAGGTGCA CCATGAACAG GTAGTCGCGC GCGCGTCGGA

23001  CCATGCCCTT CTCCCACGCA GACACGATCG GCACACTCAG CGGGTTCATC
       GGTACGGGAA GAGGGTGCGT CTGTGCTAGC CGTGTGAGTC GCCCAAGTAG

23051  ACCGTAATTT CACTTTCCGC TTCGCTGGGC TCTTCCTCTT CCTCTTGCGT
       TGGCATTAAA GTGAAAGGCG AAGCGACCCG AGAAGGAGAA GGAGAACGCA

23101  CCGCATACCA CGCGCCACTG GGTCGTCTTC ATTCAGCCGC CGCACTGTGC
       GGCGTATGGT GCGCGGTGAC CCAGCAGAAG TAAGTCGGCG GCGTGACACG

23151  GCTTACCTCC TTTGCCATGC TTGATTAGCA CCGGTGGGTT GCTGAAACCC
       CGAATGGAGG AAACGGTACG AACTAATCGT GGCCACCCAA CGACTTTGGG

23201  ACCATTTGTA GCGCCACATC TTCTCTTTCT TCCTCGCTGT CCACGATTAC
       TGGTAAACAT CGCGGTGTAG AAGAGAAAGA AGGAGCGACA GGTGCTAATG

23251  CTCTGGTGAT GGCGGGCGCT CGGGCTTGGG AGAAGGGCGC TTCTTTTTCT
       GAGACCACTA CCGCCCGCGA GCCCGAACCC TCTTCCCGCG AAGAAAAAGA

23301  TCTTGGGCGC AATGGCCAAA TCCGCCGCCG AGGTCGATGG CCGCGGGCTG
       AGAACCCGCG TTACCGGTTT AGGCGGCGGC TCCAGCTACC GGCGCCCGAC

23351  GGTGTGCGCG GCACCAGCGC GTCTTGTGAT GAGTCTTCCT CGTCCTCGGA
       CCACACGCGC CGTGGTCGCG CAGAACACTA CTCAGAAGGA GCAGGAGCCT

23401  CTCGATACGC CGCCTCATCC GCTTTTTTGG GGGCGCCCGG GGAGGCGGCG
       GAGCTATGCG GCGGAGTAGG CGAAAAAACC CCCGCGGGCC CCTCCGCCGC

23451  GCGACGGGGA CGGGGACGAC ACGTCCTCCA TGGTTGGGGG ACGTCGCGCC
       CGCTGCCCCT GCCCCTGCTG TGCAGGAGGT ACCAACCCCC TGCAGCGCGG

23501  GCACCGCGTC CGCGCTCGGG GGTGGTTTCG CGCTGCTCCT CTTCCCGACT
       CGTGGCGCAG GCGCGAGCCC CCACCAAAGC GCGACGAGGA GAAGGGCTGA

23551  GGCCATTTCC TTCTCCTATA GGCAGAAAAA GATCATGGAG TCAGTCGAGA
       CCGGTAAAGG AAGAGGATAT CCGTCTTTTT CTAGTACCTC AGTCAGCTCT

23601  AGAAGGACAG CCTAACCGCC CCCTCTGAGT TCGCCACCAC CGCCTCCACC
       TCTTCCTGTC GGATTGGCGG GGGAGACTCA AGCGGTGGTG GCGGAGGTGG

23651  GATGCCGCCA ACGCGCCTAC CACCTTCCCC GTCGAGGCAC CCCCGCTTGA
       CTACGGCGGT TGCGCGGATG GTGGAAGGGG CAGCTCCGTG GGGGCGAACT

23701  GGAGGAGGAA GTGATTATCG AGCAGGACCC AGGTTTTGTA AGCGAAGACG
       CCTCCTCCTT CACTAATAGC TCGTCCTGGG TCCAAAACAT TCGCTTCTGC
```

FIG.9A-28

```
23751  ACGAGGACCG CTCAGTACCA ACAGAGGATA AAAAGCAAGA CCAGGACAAC
       TGCTCCTGGC GAGTCATGGT TGTCTCCTAT TTTTCGTTCT GGTCCTGTTG

23801  GCAGAGGCAA ACGAGGAACA AGTCGGGCGG GGGGACGAAA GGCATGGCGA
       CGTCTCCGTT TGCTCCTTGT TCAGCCGCC CCCCTGCTTT CCGTACCGCT

23851  CTACCTAGAT GTGGGAGACG ACGTGCTGTT GAAGCATCTG CAGCGCCAGT
       GATGGATCTA CACCCTCTGC TGCACGACAA CTTCGTAGAC GTCGCGGTCA

23901  GCGCCATTAT CTGCGACGCG TTGCAAGAGC GCAGCGATGT GCCCCTCGCC
       CGCGGTAATA GACGCTGCGC AACGTTCTCG CGTCGCTACA CGGGGAGCGG

23951  ATAGCGGATG TCAGCCTTGC CTACGAACGC CACCTATTCT CACCGCGCGT
       TATCGCCTAC AGTCGGAACG GATGCTTGCG GTGGATAAGA GTGGCGCGCA

24001  ACCCCCCAAA CGCCAAGAAA ACGGCACATG CGAGCCCAAC CCGCGCCTCA
       TGGGGGGTTT GCGGTTCTTT TGCCGTGTAC GCTCGGGTTG GGCGCGGAGT

24051  ACTTCTACCC CGTATTTGCC GTGCCAGAGG TGCTTGCCAC CTATCACATC
       TGAAGATGGG GCATAAACGG CACGGTCTCC ACGAACGGTG GATAGTGTAG

24101  TTTTTCCAAA ACTGCAAGAT ACCCCTATCC TGCCGTGCCA ACCGCAGCCG
       AAAAAGGTTT TGACGTTCTA TGGGGATAGG ACGGCACGGT TGGCGTCGGC

24151  AGCGGACAAG CAGCTGGCCT TGCGGCAGGG CGCTGTCATA CCTGATATCG
       TCGCCTGTTC GTCGACCGGA ACGCCGTCCC GCGACAGTAT GGACTATAGC

24201  CCTCGCTCAA CGAAGTGCCA AAAATCTTTG AGGGTCTTGG ACGCGACGAG
       GGAGCGAGTT GCTTCACGGT TTTTAGAAAC TCCCAGAACC TGCGCTGCTC

24251  AAGCGCGCGG CAAACGCTCT GCAACAGGAA AACAGCGAAA ATGAAAGTCA
       TTCGCGCGCC GTTTGCGAGA CGTTGTCCTT TTGTCGCTTT TACTTTCAGT

24301  CTCTGGAGTG TTGGTGGAAC TCGAGGGTGA CAACGCGCGC CTAGCCGTAC
       GAGACCTCAC AACCACCTTG AGCTCCCACT GTTGCGCGCG GATCGGCATG

24351  TAAAACGCAG CATCGAGGTC ACCCACTTTG CCTACCCGGC ACTTAACCTA
       ATTTTGCGTC GTAGCTCCAG TGGGTGAAAC GGATGGGCCG TGAATTGGAT

24401  CCCCCCAAGG TCATGAGCAC AGTCATGAGT GAGCTGATCG TGCGCCGTGC
       GGGGGGTTCC AGTACTCGTG TCAGTACTCA CTCGACTAGC ACGCGGCACG

24451  GCAGCCCCTG GAGAGGGATG CAAATTTGCA AGAACAAACA GAGGAGGGCC
       CGTCGGGGAC CTCTCCCTAC GTTTAAACGT TCTTGTTTGT CTCCTCCCGG

24501  TACCCGCAGT TGGCGACGAG CAGCTAGCGC GCTGGCTTCA AACGCGCGAG
       ATGGGCGTCA ACCGCTGCTC GTCGATCGCG CGACCGAAGT TTGCGCGCTC

24551  CCTGCCGACT TGGAGGAGCG ACGCAAACTA ATGATGGCCG CAGTGCTCGT
       GGACGGCTGA ACCTCCTCGC TGCGTTTGAT TACTACCGGC GTCACGAGCA
```

FIG.9A-29

```
24601  TACCGTGGAG CTTGAGTGCA TGCAGCGGTT CTTTGCTGAC CCGGAGATGC
       ATGGCACCTC GAACTCACGT ACGTCGCCAA GAAACGACTG GGCCTCTACG

24651  AGCGCAAGCT AGAGGAAACA TTGCACTACA CCTTTCGACA GGGCTACGTA
       TCGCGTTCGA TCTCCTTTGT AACGTGATGT GGAAAGCTGT CCCGATGCAT

24701  CGCCAGGCCT GCAAGATCTC CAACGTGGAG CTCTGCAACC TGGTCTCCTA
       GCGGTCCGGA CGTTCTAGAG GTTGCACCTC GAGACGTTGG ACCAGAGGAT

24751  CCTTGGAATT TTGCACGAAA ACCGCCTTGG GCAAAACGTG CTTCATTCCA
       GGAACCTTAA AACGTGCTTT TGGCGGAACC CGTTTTGCAC GAAGTAAGGT

24801  CGCTCAAGGG CGAGGCGCGC CGCGACTACG TCCGCGACTG CGTTTACTTA
       GCGAGTTCCC GCTCCGCGCG GCGCTGATGC AGGCGCTGAC GCAAATGAAT

24851  TTTCTATGCT ACACCTGGCA GACGGCCATG GGCGTTTGGC AGCAGTGCTT
       AAAGATACGA TGTGGACCGT CTGCCGGTAC CCGCAAACCG TCGTCACGAA

24901  GGAGGAGTGC AACCTCAAGG AGCTGCAGAA ACTGCTAAAG CAAAACTTGA
       CCTCCTCACG TTGGAGTTCC TCGACGTCTT TGACGATTTC GTTTTGAACT

24951  AGGACCTATG GACGGCCTTC AACGAGCGCT CCGTGGCCGC GCACCTGGCG
       TCCTGGATAC CTGCCGGAAG TTGCTCGCGA GGCACCGGCG CGTGGACCGC

25001  GACATCATTT TCCCCGAACG CCTGCTTAAA ACCCTGCAAC AGGGTCTGCC
       CTGTAGTAAA AGGGGCTTGC GGACGAATTT TGGGACGTTG TCCCAGACGG

25051  AGACTTCACC AGTCAAAGCA TGTTGCAGAA CTTTAGGAAC TTTATCCTAG
       TCTGAAGTGG TCAGTTTCGT ACAACGTCTT GAAATCCTTG AAATAGGATC

25101  AGCGCTCAGG AATCTTGCCC GCCACCTGCT GTGCACTTCC TAGCGACTTT
       TCGCGAGTCC TTAGAACGGG CGGTGGACGA CACGTGAAGG ATCGCTGAAA

25151  GTGCCCATTA AGTACCGCGA ATGCCCTCCG CCGCTTTGGG GCCACTGCTA
       CACGGGTAAT TCATGGCGCT TACGGGAGGC GGCGAAACCC CGGTGACGAT

25201  CCTTCTGCAG CTAGCCAACT ACCTTGCCTA CCACTCTGAC ATAATGGAAG
       GGAAGACGTC GATCGGTTGA TGGAACGGAT GGTGAGACTG TATTACCTTC

25251  ACGTGAGCGG TGACGGTCTA CTGGAGTGTC ACTGTCGCTG CAACCTATGC
       TGCACTCGCC ACTGCCAGAT GACCTCACAG TGACAGCGAC GTTGGATACG

25301  ACCCCGCACC GCTCCCTGGT TTGCAATTCG CAGCTGCTTA ACGAAAGTCA
       TGGGGCGTGG CGAGGGACCA AACGTTAAGC GTCGACGAAT TGCTTTCAGT

25351  AATTATCGGT ACCTTTGAGC TGCAGGGTCC CTCGCCTGAC GAAAAGTCCG
       TTAATAGCCA TGGAAACTCG ACGTCCCAGG GAGCGGACTG CTTTTCAGGC

25401  CGGCTCCGGG GTTGAAACTC ACTCCGGGGC TGTGGACGTC GGCTTACCTT
       GCCGAGGCCC CAACTTTGAG TGAGGCCCCG ACACCTGCAG CCGAATGGAA
```

FIG.9A-30

```
25451  CGCAAATTTG TACCTGAGGA CTACCACGCC CACGAGATTA GGTTCTACGA
       GCGTTTAAAC ATGGACTCCT GATGGTGCGG GTGCTCTAAT CCAAGATGCT

25501  AGACCAATCC CGCCCGCCTA ATGCGGAGCT TACCGCCTGC GTCATTACCC
       TCTGGTTAGG GCGGGCGGAT TACGCCTCGA ATGGCGGACG CAGTAATGGG

25551  AGGGCCACAT TCTTGGCCAA TTGCAAGCCA TCAACAAAGC CCGCCAAGAG
       TCCCGGTGTA AGAACCGGTT AACGTTCGGT AGTTGTTTCG GGCGGTTCTC

25601  TTTCTGCTAC GAAAGGGACG GGGGGTTTAC TTGGACCCCC AGTCCGGCGA
       AAAGACGATG CTTTCCCTGC CCCCCAAATG AACCTGGGGG TCAGGCCGCT

25651  GGAGCTCAAC CCAATCCCCC CGCCGCCGCA GCCCTATCAG CAGCAGCCGC
       CCTCGAGTTG GGTTAGGGGG GCGGCGGCGT CGGGATAGTC GTCGTCGGCG

25701  GGGCCCTTGC TTCCCAGGAT GGCACCCAAA AAGAAGCTGC AGCTGCCGCC
       CCCGGGAACG AAGGGTCCTA CCGTGGGTTT TTCTTCGACG TCGACGGCGG

25751  GCCACCCACG GACGAGGAGG AATACTGGGA CAGTCAGGCA GAGGAGGTTT
       CGGTGGGTGC CTGCTCCTCC TTATGACCCT GTCAGTCCGT CTCCTCCAAA

25801  TGGACGAGGA GGAGGAGGAC ATGATGGAAG ACTGGGAGAG CCTAGACGAG
       ACCTGCTCCT CCTCCTCCTG TACTACCTTC TGACCCTCTC GGATCTGCTC

25851  GAAGCTTCCG AGGTCGAAGA GGTGTCAGAC GAAACACCGT CACCCTCGGT
       CTTCGAAGGC TCCAGCTTCT CCACAGTCTG CTTTGTGGCA GTGGGAGCCA

25901  CGCATTCCCC TCGCCGGCGC CCCAGAAATC GGCAACCGGT TCCAGCATGG
       GCGTAAGGGG AGCGGCCGCG GGGTCTTTAG CCGTTGGCCA AGGTCGTACC

25951  CTACAACCTC CGCTCCTCAG GCGCCGCCGG CACTGCCCGT TCGCCGACCC
       GATGTTGGAG GCGAGGAGTC CGCGGCGGCC GTGACGGGCA AGCGGCTGGG

26001  AACCGTAGAT GGGACACCAC TGGAACCAGG GCCGGTAAGT CCAAGCAGCC
       TTGGCATCTA CCCTGTGGTG ACCTTGGTCC CGGCCATTCA GGTTCGTCGG

26051  GCCGCCGTTA GCCCAAGAGC AACAACAGCG CCAAGGCTAC CGCTCATGGC
       CGGCGGCAAT CGGGTTCTCG TTGTTGTCGC GGTTCCGATG GCGAGTACCG

26101  GCGGGCACAA GAACGCCATA GTTGCTTGCT TGCAAGACTG TGGGGGCAAC
       CGCCCGTGTT CTTGCGGTAT CAACGAACGA ACGTTCTGAC ACCCCCGTTG

26151  ATCTCCTTCG CCCGCCGCTT TCTTCTCTAC CATCACGGCG TGGCCTTCCC
       TAGAGGAAGC GGGCGGCGAA AGAAGAGATG GTAGTGCCGC ACCGGAAGGG

26201  CCGTAACATC CTGCATTACT ACCGTCATCT CTACAGCCCA TACTGCACCG
       GGCATTGTAG GACGTAATGA TGGCAGTAGA GATGTCGGGT ATGACGTGGC

26251  GCGGCAGCGG CAGCAACAGC AGCGGCCACA CAGAAGCAAA GGCGACCGGA
       CGCCGTCGCC GTCGTTGTCG TCGCCGGTGT GTCTTCGTTT CCGCTGGCCT
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FIG.9A-31

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26301  TAGCAAGACT CTGACAAAGC CCAAGAAATC CACAGCGGCG GCAGCAGCAG
       ATCGTTCTGA GACTGTTTCG GGTTCTTTAG GTGTCGCCGC CGTCGTCGTC

26351  GAGGAGGAGC GCTGCGTCTG GCGCCCAACG AACCCGTATC GACCCGCGAG
       CTCCTCCTCG CGACGCAGAC CGCGGGTTGC TTGGGCATAG CTGGGCGCTC

26401  CTTAGAAACA GGATTTTTCC CACTCTGTAT GCTATATTTC AACAGAGCAG
       GAATCTTTGT CCTAAAAAGG GTGAGACATA CGATATAAAG TTGTCTCGTC

26451  GGGCCAAGAA CAAGAGCTGA AAATAAAAAA CAGGTCTCTG CGATCCCTCA
       CCCGGTTCTT GTTCTCGACT TTTATTTTTT GTCCAGAGAC GCTAGGGAGT

26501  CCCGCAGCTG CCTGTATCAC AAAAGCGAAG ATCAGCTTCG GCGCACGCTG
       GGGCGTCGAC GGACATAGTG TTTTCGCTTC TAGTCGAAGC CGCGTGCGAC

26551  GAAGACGCGG AGGCTCTCTT CAGTAAATAC TGCGCGCTGA CTCTTAAGGA
       CTTCTGCGCC TCCGAGAGAA GTCATTTATG ACGCGCGACT GAGAATTCCT

26601  CTAGTTTCGC GCCCTTTCTC AAATTTAAGC GCGAAAACTA CGTCATCTCC
       GATCAAAGCG CGGGAAAGAG TTTAAATTCG CGCTTTTGAT GCAGTAGAGG

26651  AGCGGCCACA CCCGGCGCCA GCACCTGTTG TCAGCGCCAT TATGAGCAAG
       TCGCCGGTGT GGGCCGCGGT CGTGGACAAC AGTCGCGGTA ATACTCGTTC

26701  GAAATTCCCA CGCCCTACAT GTGGAGTTAC CAGCCACAAA TGGGACTTGC
       CTTTAAGGGT GCGGGATGTA CACCTCAATG GTCGGTGTTT ACCCTGAACG

26751  GGCTGGAGCT GCCCAAGACT ACTCAACCCG AATAAACTAC ATGAGCGCGG
       CCGACCTCGA CGGGTTCTGA TGAGTTGGGC TTATTTGATG TACTCGCGCC

26801  GACCCCACAT GATATCCCGG GTCAACGGAA TACGCGCCCA CCGAAACCGA
       CTGGGGTGTA CTATAGGGCC CAGTTGCCTT ATGCGCGGGT GGCTTTGGCT

26851  ATTCTCCTGG AACAGGCGGC TATTACCACC ACACCTCGTA ATAACCTTAA
       TAAGAGGACC TTGTCCGCCG ATAATGGTGG TGTGGAGCAT TATTGGAATT

26901  TCCCCGTAGT TGGCCCGCTG CCCTGGTGTA CCAGGAAAGT CCCGCTCCCA
       AGGGGCATCA ACCGGGCGAC GGGACCACAT GGTCCTTTCA GGGCGAGGGT

26951  CCACTGTGGT ACTTCCCAGA GACGCCCAGG CCGAAGTTCA GATGACTAAC
       GGTGACACCA TGAAGGGTCT CTGCGGGTCC GGCTTCAAGT CTACTGATTG

27001  TCAGGGGCGC AGCTTGCGGG CGGCTTTCGT CACAGGGTGC GGTCGCCCGG
       AGTCCCCGCG TCGAACGCCC GCCGAAAGCA GTGTCCCACG CCAGCGGGCC

27051  GCAGGGTATA ACTCACCTGA CAATCAGAGG GCGAGGTATT CAGCTCAACG
       CGTCCCATAT TGAGTGGACT GTTAGTCTCC CGCTCCATAA GTCGAGTTGC

27101  ACGAGTCGGT GAGCTCCTCG CTTGGTCTCC GTCCGGACGG GACATTTCAG
       TGCTCAGCCA CTCGAGGAGC GAACCAGAGG CAGGCCTGCC CTGTAAAGTC
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FIG.9A-32

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27151  ATCGGCGGCG CCGGCCGCTC TTCATTCACG CCTCGTCAGG CAATCCTAAC
       TAGCCGCCGC GGCCGGCGAG AAGTAAGTGC GGAGCAGTCC GTTAGGATTG

27201  TCTGCAGACC TCGTCCTCTG AGCCGCGCTC TGGAGGCATT GGAACTCTGC
       AGACGTCTGG AGCAGGAGAC TCGGCGCGAG ACCTCCGTAA CCTTGAGACG

27251  AATTTATTGA GGAGTTTGTG CCATCGGTCT ACTTTAACCC CTTCTCGGGA
       TTAAATAACT CCTCAAACAC GGTAGCCAGA TGAAATTGGG GAAGAGCCCT

27301  CCTCCCGGCC ACTATCCGGA TCAATTTATT CCTAACTTTG ACGCGGTAAA
       GGAGGGCCGG TGATAGGCCT AGTTAAATAA GGATTGAAAC TGCGCCATTT

27351  GGACTCGGCG GACGGCTACG ACTGAATGTT AAGTGGAGAG GCAGAGCAAC
       CCTGAGCCGC CTGCCGATGC TGACTTACAA TTCACCTCTC CGTCTCGTTG

27401  TGCGCCTGAA ACACCTGGTC CACTGTCGCC GCCACAAGTG CTTTGCCCGC
       ACGCGGACTT TGTGGACCAG GTGACAGCGG CGGTGTTCAC GAAACGGGCG

27451  GACTCCGGTG AGTTTTGCTA CTTTGAATTG CCCGAGGATC ATATCGAGGG
       CTGAGGCCAC TCAAAACGAT GAAACTTAAC GGGCTCCTAG TATAGCTCCC

27501  CCCGGCGCAC GGCGTCCGGC TTACCGCCCA GGGAGAGCTT GCCCGTAGCC
       GGGCCGCGTG CCGCAGGCCG AATGGCGGGT CCCTCTCGAA CGGGCATCGG

27551  TGATTCGGGA GTTTACCCAG CGCCCCCTGC TAGTTGAGCG GGACAGGGGA
       ACTAAGCCCT CAAATGGGTC GCGGGGGACG ATCAACTCGC CCTGTCCCCT

27601  CCCTGTGTTC TCACTGTGAT TTGCAACTGT CCTAACCCTG GATTACATCA
       GGGACACAAG AGTGACACTA AACGTTGACA GGATTGGGAC CTAATGTAGT

27651  AGATCTTTGT TGCCATCTCT GTGCTGAGTA TAATAAATAC AGAAATTAAA
       TCTAGAAACA ACGGTAGAGA CACGACTCAT ATTATTTATG TCTTTAATTT

27701  ATATACTGGG GCTCCTATCG CCATCCTGTA AACGCCACCG TCTTCACCCG
       TATATGACCC CGAGGATAGC GGTAGGACAT TTGCGGTGGC AGAAGTGGGC

27751  CCCAAGCAAA CCAAGGCGAA CCTTACCTGG TACTTTTAAC ATCTCTCCCT
       GGGTTCGTTT GGTTCCGCTT GGAATGGACC ATGAAAATTG TAGAGAGGGA

27801  CTGTGATTTA CAACAGTTTC AACCCAGACG GAGTGAGTCT ACGAGAGAAC
       GACACTAAAT GTTGTCAAAG TTGGGTCTGC CTCACTCAGA TGCTCTCTTG

27851  CTCTCCGAGC TCAGCTACTC CATCAGAAAA AACACCACCC TCCTTACCTG
       GAGAGGCTCG AGTCGATGAG GTAGTCTTTT TTGTGGTGGG AGGAATGGAC

27901  CCGGGAACGT ACGAGTGCGT CACCGGCCGC TGCACCACAC CTACCGCCTG
       GGCCCTTGCA TGCTCACGCA GTGGCCGGCG ACGTGGTGTG GATGGCGGAC

27951  ACCGTAAACC AGACTTTTTC CGGACAGACC TCAATAACTC TGTTTACCAG
       TGGCATTTGG TCTGAAAAAG GCCTGTCTGG AGTTATTGAG ACAAATGGTC
```

FIG.9A-33

28001 AACAGGAGGT GAGCTTAGAA AACCCTTAGG GTATTAGGCC AAAGGCGCAG
TTGTCCTCCA CTCGAATCTT TTGGGAATCC CATAATCCGG TTTCCGCGTC

28051 CTACTGTGGG GTTTATGAAC AATTCAAGCA ACTCTACGGG CTATTCTAAT
GATGACACCC CAAATACTTG TTAAGTTCGT TGAGATGCCC GATAAGATTA

28101 TCAGGTTTCT CTAGAATCGG GGTTGGGGTT ATTCTCTGTC TTGTGATTCT
AGTCCAAAGA GATCTTAGCC CCAACCCCAA TAAGAGACAG AACACTAAGA

28151 CTTTATTCTT ATACTAACGC TTCTCTGCCT AAGGCTCGCC GCCTGCTGTG
GAAATAAGAA TATGATTGCG AAGAGACGGA TTCCGAGCGG CGGACGACAC

28201 TGCACATTTG CATTTTATTGT CAGCTTTTTA AACGCTGGGG TCGCCACCCA
ACGTGTAAAC GTAAATAACA GTCGAAAAAT TTGCGACCCC AGCGGTGGGT

28251 AGATGATTAG GTACATAATC CTAGGTTTAC TCACCCTTGC GTCAGCCCAC
TCTACTAATC CATGTATTAG GATCCAAATG AGTGGGAACG CAGTCGGGTG

28301 GGTACCACCC AAAAGGTGGA TTTTAAGGAG CCAGCCTGTA ATGTTACATT
CCATGGTGGG TTTTCCACCT AAAATTCCTC GGTCGGACAT TACAATGTAA

28351 CGCAGCTGAA GCTAATGAGT GCACCACTCT TATAAAATGC ACCACAGAAC
GCGTCGACTT CGATTACTCA CGTGGTGAGA ATATTTTACG TGGTGTCTTG

28401 ATGAAAAGCT GCTTATTCGC CACAAAAACA AAATTGGCAA GTATGCTGTT
TACTTTTCGA CGAATAAGCG GTGTTTTTGT TTTAACCGTT CATACGACAA

28451 TATGCTATTT GGCAGCCAGG TGACACTACA GAGTATAATG TTACAGTTTT
ATACGATAAA CCGTCGGTCC ACTGTGATGT CTCATATTAC AATGTCAAAA

28501 CCAGGGTAAA AGTCATAAAA CTTTTATGTA TACTTTTCCA TTTTATGAAA
GGTCCCATTT TCAGTATTTT GAAAATACAT ATGAAAAGGT AAAATACTTT

28551 TGTGCGACAT TACCATGTAC ATGAGCAAAC AGTATAAGTT GTGGCCCCCA
ACACGCTGTA ATGGTACATG TACTCGTTTG TCATATTCAA CACCGGGGGT

28601 CAAAATTGTG TGGAAAACAC TGGCACTTTC TGCTGCACTG CTATGCTAAT
GTTTTAACAC ACCTTTTGTG ACCGTGAAAG ACGACGTGAC GATACGATTA

28651 TACAGTGCTC GCTTTGGTCT GTACCCTACT CTATATTAAA TACAAAAGCA
ATGTCACGAG CGAAACCAGA CATGGGATGA GATATAATTT ATGTTTTCGT

28701 GACGCAGCTT TATTGAGGAA AAGAAAATGC CTTAATTTAC TAAGTTACAA
CTGCGTCGAA ATAACTCCTT TTCTTTTACG GAATTAAATG ATTCAATGTT

28751 AGCTAATGTC ACCACTAACT GCTTTACTCG CTGCTTGCAA AACAAATTCA
TCGATTACAG TGGTGATTGA CGAAATGAGC GACGAACGTT TTGTTTAAGT

28801 AAAAGTTAGC ATTATAATTA GAATAGGATT TAAACCCCCC GGTCATTTCC
TTTTCAATCG TAATATTAAT CTTATCCTAA ATTTGGGGGG CCAGTAAAGG

FIG.9A-34

28851 TGCTCAATAC CATTCCCCTG AACAATTGAC TCTATGTGGG ATATGCTCCA
ACGAGTTATG GTAAGGGGAC TTGTTAACTG AGATACACCC TATACGAGGT

28901 GCGCTACAAC CTTGAAGTCA GGCTTCCTGG ATGTCAGCAT CTGACTTTGG
CGCGATGTTG GAACTTCAGT CCGAAGGACC TACAGTCGTA GACTGAAACC

28951 CCAGCACCTG TCCCGCGGAT TTGTTCCAGT CCAACTACAG CGACCCACCC
GGTCGTGGAC AGGGCGCCTA AACAAGGTCA GGTTGATGTC GCTGGGTGGG

29001 TAACAGAGAT GACCAACACA ACCAACGCGG CCGCCGCTAC CGGACTTACA
ATTGTCTCTA CTGGTTGTGT TGGTTGCGCC GGCGGCGATG GCCTGAATGT

29051 TCTACCACAA ATACACCCCA AGTTTCTGCC TTTGTCAATA ACTGGGATAA
AGATGGTGTT TATGTGGGGT TCAAAGACGG AAACAGTTAT TGACCCTATT

29101 CTTGGGCATG TGGTGGTTCT CCATAGCGCT TATGTTTGTA TGCCTTATTA
GAACCCGTAC ACCACCAAGA GGTATCGCGA ATACAAACAT ACGGAATAAT

29151 TTATGTGGCT CATCTGCTGC CTAAAGCGCA AACGCGCCCG ACCACCCATC
AATACACCGA GTAGACGACG GATTTCGCGT TTGCGCGGGC TGGTGGGTAG

29201 TATAGTCCCA TCATTGTGCT ACACCCAAAC AATGATGGAA TCCATAGATT
ATATCAGGGT AGTAACACGA TGTGGGTTTG TTACTACCTT AGGTATCTAA

29251 GGACGGACTG AAACACATGT TCTTTTCTCT TACAGTATGA TTAAATGAGA
CCTGCCTGAC TTTGTGTACA AGAAAAGAGA ATGTCATACT AATTTACTCT

29301 CATGATTCCT CGAGTTTTTA TATTACTGAC CCTTGTTGCG CTTTTTTGTG
GTACTAAGGA GCTCAAAAAT ATAATGACTG GGAACAACGC GAAAAAACAC

29351 CGTGCTCCAC ATTGGCTGCG GTTTCTCACA TCGAAGTAGA CTGCATTCCA
GCACGAGGTG TAACCGACGC CAAAGAGTGT AGCTTCATCT GACGTAAGGT

29401 GCCTTCACAG TCTATTTGCT TTACGGATTT GTCACCCTCA CGCTCATCTG
CGGAAGTGTC AGATAAACGA AATGCCTAAA CAGTGGGAGT GCGAGTAGAC

29451 CAGCCTCATC ACTGTGGTCA TCGCCTTTAT CCAGTGCATT GACTGGGTCT
GTCGGAGTAG TGACACCAGT AGCGGAAATA GGTCACGTAA CTGACCCAGA

29501 GTGTGCGCTT TGCATATCTC AGACACCATC CCCAGTACAG GGACAGGACT
CACACGCGAA ACGTATAGAG TCTGTGGTAG GGGTCATGTC CCTGTCCTGA

29551 ATAGCTGAGC TTCTTAGAAT TCTTTAATTA TGAAATTTAC TGTGACTTTT
TATCGACTCG AAGAATCTTA AGAAATTAAT ACTTTAAATG ACACTGAAAA

29601 CTGCTGATTA TTTGCACCCT ATCTGCGTTT TGTTCCCCGA CCTCCAAGCC
GACGACTAAT AAACGTGGGA TAGACGCAAA ACAAGGGGCT GGAGGTTCGG

29651 TCAAAGACAT ATATCATGCA GATTCACTCG TATATGGAAT ATTCCAAGTT
AGTTTCTGTA TATAGTACGT CTAAGTGAGC ATATACCTTA TAAGGTTCAA

FIG.9A-35

29701 GCTACAATGA AAAAAGCGAT CTTTCCGAAG CCTGGTTATA TGCAATCATC
CGATGTTACT TTTTTCGCTA GAAAGGCTTC GGACCAATAT ACGTTAGTAG

29751 TCTGTTATGG TGTTCTGCAG TACCATCTTA GCCCTAGCTA TATATCCCTA
AGACAATACC ACAAGACGTC ATGGTAGAAT CGGGATCGAT ATATAGGGAT

29801 CCTTGACATT GGCTGGAACG CAATAGATGC CATGAACCAC CCAACTTTCC
GGAACTGTAA CCGACCTTGC GTTATCTACG GTACTTGGTG GGTTGAAAGG

29851 CCGCGCCCGC TATGCTTCCA CTGCAACAAG TTGTTGCCGG CGGCTTTGTC
GGCGCGGGCG ATACGAAGGT GACGTTGTTC AACAACGGCC GCCGAAACAG

29901 CCAGCCAATC AGCCTCGCCC ACCTTCTCCC ACCCCCACTG AAATCAGCTA
GGTCGGTTAG TCGGAGCGGG TGGAAGAGGG TGGGGGTGAC TTTAGTCGAT

29951 CTTTAATCTA ACAGGAGGAG ATGACTGACA CCCTAGATCT AGAAATGGAC
GAAATTAGAT TGTCCTCCTC TACTGACTGT GGGATCTAGA TCTTTACCTG

30001 GGAATTATTA CAGAGCAGCG CCTGCTAGAA AGACGCAGGG CAGCGGCCGA
CCTTAATAAT GTCTCGTCGC GGACGATCTT TCTGCGTCCC GTCGCCGGCT

30051 GCAACAGCGC ATGAATCAAG AGCTCCAAGA CATGGTTAAC TTGCACCAGT
CGTTGTCGCG TACTTAGTTC TCGAGGTTCT GTACCAATTG AACGTGGTCA

30101 GCAAAAGGGG TATCTTTTGT CTCGTAAAGC AGGCCAAAGT CACCTACGAC
CGTTTTCCCC ATAGAAAACA GAGCATTTCG TCCGGTTTCA GTGGATGCTG

30151 AGTAATACCA CCGGACACCG CCTTAGCTAC AAGTTGCCAA CCAAGCGTCA
TCATTATGGT GGCCTGTGGC GGAATCGATG TTCAACGGTT GGTTCGCAGT

30201 GAAATTGGTG GTCATGGTGG GAGAAAAGCC CATTACCATA ACTCAGCACT
CTTTAACCAC CAGTACCACC CTCTTTTCGG GTAATGGTAT TGAGTCGTGA

30251 CGGTAGAAAC CGAAGGCTGC ATTCACTCAC CTTGTCAAGG ACCTGAGGAT
GCCATCTTTG GCTTCCGACG TAAGTGAGTG GAACAGTTCC TGGACTCCTA

30301 CTCTGCACCC TTATTAAGAC CCTGTGCGGT CTCAAAGATC TTATTCCCTT
GAGACGTGGG AATAATTCTG GGACACGCCA GAGTTTCTAG AATAAGGGAA

30351 TAACTAATAA AAAAAAATAA TAAAGCATCA CTTACTTAAA ATCAGTTAGC
ATTGATTATT TTTTTTTATT ATTTCGTAGT GAATGAATTT TAGTCAATCG

30401 AAATTTCTGT CCAGTTTATT CAGCAGCACC TCCTTGCCCT CCTCCCAGCT
TTTAAAGACA GGTCAAATAA GTCGTCGTGG AGGAACGGGA GGAGGGTCGA

30451 CTGGTATTGC AGCTTCCTCC TGGCTGCAAA CTTTCTCCAC AATCTAAATG
GACCATAACG TCGAAGGAGG ACCGACGTTT GAAAGAGGTG TTAGATTTAC

30501 GAATGTCAGT TTCCTCCTGT TCCTGTCCAT CCGCACCCAC TATCTTCATG
CTTACAGTCA AAGGAGGACA AGGACAGGTA GGCGTGGGTG ATAGAAGTAC

FIG.9A-36

30551 TTGTTGCAGA TGAAGCGCGC AAGACCGTCT GAAGATACCT TCAACCCCGT
AACAACGTCT ACTTCGCGCG TTCTGGCAGA CTTCTATGGA AGTTGGGGCA

30601 GTATCCATAT GACACGGAAA CCGGTCCTCC AACTGTGCCT TTTCTTACTC
CATAGGTATA CTGTGCCTTT GGCCAGGAGG TTGACACGGA AAAGAATGAG

30651 CTCCCTTTGT ATCCCCCAAT GGGTTTCAAG AGAGTCCCCC TGGGGTACTC
GAGGGAAACA TAGGGGGTTA CCCAAAGTTC TCTCAGGGGG ACCCCATGAG

30701 TCTTTGCGCC TATCCGAACC TCTAGTTACC TCCAATGGCA TGCTTGCGCT
AGAAACGCGG ATAGGCTTGG AGATCAATGG AGGTTACCGT ACGAACGCGA

30751 CAAAATGGGC AACGGCCTCT CTCTGGACGA GGCCGGCAAC CTTACCTCCC
GTTTTACCCG TTGCCGGAGA GAGACCTGCT CCGGCCGTTG GAATGGAGGG

30801 AAAATGTAAC CACTGTGAGC CCACCTCTCA AAAAAACCAA GTCAAACATA
TTTTACATTG GTGACACTCG GGTGGAGAGT TTTTTTGGTT CAGTTTGTAT

30851 AACCTGGAAA TATCTGCACC CCTCACAGTT ACCTCAGAAG CCCTAACTGT
TTGGACCTTT ATAGACGTGG GGAGTGTCAA TGGAGTCTTC GGGATTGACA

30901 GGCTGCCGCC GCACCTCTAA TGGTCGCGGG CAACACACTC ACCATGCAAT
CCGACGGCGG CGTGGAGATT ACCAGCGCCC GTTGTGTGAG TGGTACGTTA

30951 CACAGGCCCC GCTAACCGTG CACGACTCCA AACTTAGCAT TGCCACCCAA
GTGTCCGGGG CGATTGGCAC GTGCTGAGGT TTGAATCGTA ACGGTGGGTT

31001 GGACCCCTCA CAGTGTCAGA AGGAAAGCTA GCCCTGCAAA CATCAGGCCC
CCTGGGGAGT GTCACAGTCT TCCTTTCGAT CGGGACGTTT GTAGTCCGGG

31051 CCTCACCACC ACCGATAGCA GTACCCTTAC TATCACTGCC TCACCCCCTT
GGAGTGGTGG TGGCTATCGT CATGGGAATG ATAGTGACGG AGTGGGGGAA

31101 TAACTACTGC CACTGGTAGC TTGGGCATTG ACTTGAAAGA GCCCATTTAT
ATTGATGACG GTGACCATCG AACCCGTAAC TGAACTTTCT CGGGTAAATA

31151 ACACAAAATG GAAAACTAGG ACTAAAGTAC GGGGCTCCTT TGCATGTAAC
TGTGTTTTAC CTTTTGATCC TGATTTCATG CCCCGAGGAA ACGTACATTG

31201 AGACGACCTA AACACTTTGA CCGTAGCAAC TGGTCCAGGT GTGACTATTA
TCTGCTGGAT TTGTGAAACT GGCATCGTTG ACCAGGTCCA CACTGATAAT

31251 ATAATACTTC CTTGCAAACT AAAGTTACTG GAGCCTTGGG TTTTGATTCA
TATTATGAAG GAACGTTTGA TTTCAATGAC CTCGGAACCC AAAACTAAGT

31301 CAAGGCAATA TGCAACTTAA TGTAGCAGGA GGACTAAGGA TTGATTCTCA
GTTCCGTTAT ACGTTGAATT ACATCGTCCT CCTGATTCCT AACTAAGAGT

31351 AAACAGACGC CTTATACTTG ATGTTAGTTA TCCGTTTGAT GCTCAAAACC
TTTGTCTGCG GAATATGAAC TACAATCAAT AGGCAAACTA CGAGTTTTGG

FIG.9A-37

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31401  AACTAAATCT AAGACTAGGA CAGGGCCCTC TTTTTATAAA CTCAGCCCAC
       TTGATTTAGA TTCTGATCCT GTCCCGGGAG AAAAATATTT GAGTCGGGTG

31451  AACTTGGATA TTAACTACAA CAAAGGCCTT TACTTGTTTA CAGCTTCAAA
       TTGAACCTAT AATTGATGTT GTTTCCGGAA ATGAACAAAT GTCGAAGTTT

31501  CAATTCCAAA AAGCTTGAGG TTAACCTAAG CACTGCCAAG GGGTTGATGT
       GTTAAGGTTT TTCGAACTCC AATTGGATTC GTGACGGTTC CCCAACTACA

31551  TTGACGCTAC AGCCATAGCC ATTAATGCAG GAGATGGGCT TGAATTTGGT
       AACTGCGATG TCGGTATCGG TAATTACGTC CTCTACCCGA ACTTAAACCA

31601  TCACCTAATG CACCAAACAC AAATCCCCTC AAAACAAAAA TTGGCCATGG
       AGTGGATTAC GTGGTTTGTG TTTAGGGGAG TTTTGTTTTT AACCGGTACC

31651  CCTAGAATTT GATTCAAACA AGGCTATGGT TCCTAAACTA GGAACTGGCC
       GGATCTTAAA CTAAGTTTGT TCCGATACCA AGGATTTGAT CCTTGACCGG

31701  TTAGTTTTGA CAGCACAGGT GCCATTACAG TAGGAAACAA AAATAATGAT
       AATCAAAACT GTCGTGTCCA CGGTAATGTC ATCCTTTGTT TTTATTACTA

31751  AAGCTAACTT TGTGGACCAC ACCAGCTCCA TCTCCTAACT GTAGACTAAA
       TTCGATTGAA ACACCTGGTG TGGTCGAGGT AGAGGATTGA CATCTGATTT

31801  TGCAGAGAAA GATGCTAAAC TCACTTTGGT CTTAACAAAA TGTGGCAGTC
       ACGTCTCTTT CTACGATTTG AGTGAAACCA GAATTGTTTT ACACCGTCAG

31851  AAATACTTGC TACAGTTTCA GTTTTGGCTG TTAAAGGCAG TTTGGCTCCA
       TTTATGAACG ATGTCAAAGT CAAAACCGAC AATTTCCGTC AAACCGAGGT

31901  ATATCTGGAA CAGTTCAAAG TGCTCATCTT ATTATAAGAT TTGACGAAAA
       TATAGACCTT GTCAAGTTTC ACGAGTAGAA TAATATTCTA AACTGCTTTT

31951  TGGAGTGCTA CTAAACAATT CCTTCCTGGA CCCAGAATAT TGGAACTTTA
       ACCTCACGAT GATTTGTTAA GGAAGGACCT GGGTCTTATA ACCTTGAAAT

32001  GAAATGGAGA TCTTACTGAA GGCACAGCCT ATACAAACGC TGTTGGATTT
       CTTTACCTCT AGAATGACTT CCGTGTCGGA TATGTTTGCG ACAACCTAAA

32051  ATGCCTAACC TATCAGCTTA TCCAAAATCT CACGGTAAAA CTGCCAAAAG
       TACGGATTGG ATAGTCGAAT AGGTTTTAGA GTGCCATTTT GACGGTTTTC

32101  TAACATTGTC AGTCAAGTTT ACTTAAACGG AGACAAAACT AAACCTGTAA
       ATTGTAACAG TCAGTTCAAA TGAATTTGCC TCTGTTTTGA TTTGGACATT

32151  CACTAACCAT TACACTAAAC GGTACACAGG AAACAGGAGA CACAACTCCA
       GTGATTGGTA ATGTGATTTG CCATGTGTCC TTTGTCCTCT GTGTTGAGGT

32201  AGTGCATACT CTATGTCATT TTCATGGGAC TGGTCTGGCC ACAACTACAT
       TCACGTATGA GATACAGTAA AAGTACCCTG ACCAGACCGG TGTTGATGTA
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FIG.9A-38

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32251  TAATGAAATA TTTGCCACAT CCTCTTACAC TTTTTCATAC ATTGCCCAAG
       ATTACTTTAT AAACGGTGTA GGAGAATGTG AAAAAGTATG TAACGGGTTC

32301  AATAAAGAAT CGTTTGTGTT ATGTTTCAAC GTGTTTATTT TTCAATTGCA
       TTATTTCTTA GCAAACACAA TACAAAGTTG CACAAATAAA AAGTTAACGT

32351  GAAAATTTCA AGTCATTTTT CATTCAGTAG TATAGCCCCA CCACCACATA
       CTTTTAAAGT TCAGTAAAAA GTAAGTCATC ATATCGGGGT GGTGGTGTAT

32401  GCTTATACAG ATCACCGTAC CTTAATCAAA CTCACAGAAC CCTAGTATTC
       CGAATATGTC TAGTGGCATG GAATTAGTTT GAGTGTCTTG GGATCATAAG

32451  AACCTGCCAC CTCCCTCCCA ACACACAGAG TACACAGTCC TTTCTCCCCG
       TTGGACGGTG GAGGGAGGGT TGTGTGTCTC ATGTGTCAGG AAAGAGGGGC

32501  GCTGGCCTTA AAAAGCATCA TATCATGGGT AACAGACATA TTCTTAGGTG
       CGACCGGAAT TTTTCGTAGT ATAGTACCCA TTGTCTGTAT AAGAATCCAC

32551  TTATATTCCA CACGGTTTCC TGTCGAGCCA AACGCTCATC AGTGATATTA
       AATATAAGGT GTGCCAAAGG ACAGCTCGGT TTGCGAGTAG TCACTATAAT

32601  ATAAACTCCC CGGGCAGCTC ACTTAAGTTC ATGTCGCTGT CCAGCTGCTG
       TATTTGAGGG GCCCGTCGAG TGAATTCAAG TACAGCGACA GGTCGACGAC

32651  AGCCACAGGC TGCTGTCCAA CTTGCGGTTG CTTAACGGGC GGCGAAGGAG
       TCGGTGTCCG ACGACAGGTT GAACGCCAAC GAATTGCCCG CCGCTTCCTC

32701  AAGTCCACGC CTACATGGGG GTAGAGTCAT AATCGTGCAT CAGGATAGGG
       TTCAGGTGCG GATGTACCCC CATCTCAGTA TTAGCACGTA GTCCTATCCC

32751  CGGTGGTGCT GCAGCAGCGC GCGAATAAAC TGCTGCCGCC GCCGCTCCGT
       GCCACCACGA CGTCGTCGCG CGCTTATTTG ACGACGGCGG CGGCGAGGCA

32801  CCTGCAGGAA TACAACATGG CAGTGGTCTC CTCAGCGATG ATTCGCACCG
       GGACGTCCTT ATGTTGTACC GTCACCAGAG GAGTCGCTAC TAAGCGTGGC

32851  CCCGCAGCAT AAGGCGCCTT GTCCTCCGGG CACAGCAGCG CACCCTGATC
       GGGCGTCGTA TTCCGCGGAA CAGGAGGCCC GTGTCGTCGC GTGGGACTAG

32901  TCACTTAAAT CAGCACAGTA ACTGCAGCAC AGCACCACAA TATTGTTCAA
       AGTGAATTTA GTCGTGTCAT TGACGTCGTG TCGTGGTGTT ATAACAAGTT

32951  AATCCCACAG TGCAAGGCGC TGTATCCAAA GCTCATGGCG GGGACCACAG
       TTAGGGTGTC ACGTTCCGCG ACATAGGTTT CGAGTACCGC CCCTGGTGTC

33001  AACCCACGTG GCCATCATAC CACAAGCGCA GGTAGATTAA GTGGCGACCC
       TTGGGTGCAC CGGTAGTATG GTGTTCGCGT CCATCTAATT CACCGCTGGG

33051  CTCATAAACA CGCTGGACAT AAACATTACC TCTTTTGGCA TGTTGTAATT
       GAGTATTTGT GCGACCTGTA TTTGTAATGG AGAAAACCGT ACAACATTAA
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FIG.9A-39

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33101  CACCACCTCC CGGTACCATA TAAACCTCTG ATTAAACATG GCGCCATCCA
       GTGGTGGAGG GCCATGGTAT ATTTGGAGAC TAATTTGTAC CGCGGTAGGT

33151  CCACCATCCT AAACCAGCTG GCCAAAACCT GCCCGCCGGC TATACACTGC
       GGTGGTAGGA TTTGGTCGAC CGGTTTTGGA CGGGCGGCCG ATATGTGACG

33201  AGGGAACCGG GACTGGAACA ATGACAGTGG AGAGCCCAGG ACTCGTAACC
       TCCCTTGGCC CTGACCTTGT TACTGTCACC TCTCGGGTCC TGAGCATTGG

33251  ATGGATCATC ATGCTCGTCA TGATATCAAT GTTGGCACAA CACAGGCACA
       TACCTAGTAG TACGAGCAGT ACTATAGTTA CAACCGTGTT GTGTCCGTGT

33301  CGTGCATACA CTTCCTCAGG ATTACAAGCT CCTCCCGCGT TAGAACCATA
       GCACGTATGT GAAGGAGTCC TAATGTTCGA GGAGGGCGCA ATCTTGGTAT

33351  TCCCAGGGAA CAACCCATTC CTGAATCAGC GTAAATCCCA CACTGCAGGG
       AGGGTCCCTT GTTGGGTAAG GACTTAGTCG CATTTAGGGT GTGACGTCCC

33401  AAGACCTCGC ACGTAACTCA CGTTGTGCAT TGTCAAAGTG TTACATTCGG
       TTCTGGAGCG TGCATTGAGT GCAACACGTA ACAGTTTCAC AATGTAAGCC

33451  GCAGCAGCGG ATGATCCTCC AGTATGGTAG CGCGGGTTTC TGTCTCAAAA
       CGTCGTCGCC TACTAGGAGG TCATACCATC GCGCCCAAAG ACAGAGTTTT

33501  GGAGGTAGAC GATCCCTACT GTACGGAGTG CGCCGAGACA ACCGAGATCG
       CCTCCATCTG CTAGGGATGA CATGCCTCAC GCGGCTCTGT TGGCTCTAGC

33551  TGTTGGTCGT AGTGTCATGC CAAATGGAAC GCCGGACGTA GTCATATTTC
       ACAACCAGCA TCACAGTACG GTTTACCTTG CGGCCTGCAT CAGTATAAAG

33601  CTGAAGCAAA ACCAGGTGCG GGCGTGACAA ACAGATCTGC GTCTCCGGTC
       GACTTCGTTT TGGTCCACGC CCGCACTGTT TGTCTAGACG CAGAGGCCAG

33651  TCGCCGCTTA GATCGCTCTG TGTAGTAGTT GTAGTATATC CACTCTCTCA
       AGCGGCGAAT CTAGCGAGAC ACATCATCAA CATCATATAG GTGAGAGAGT

33701  AAGCATCCAG GCGCCCCCTG GCTTCGGGTT CTATGTAAAC TCCTTCATGC
       TTCGTAGGTC CGCGGGGGAC CGAAGCCCAA GATACATTTG AGGAAGTACG

33751  GCCGCTGCCC TGATAACATC CACCACCGCA GAATAAGCCA CACCCAGCCA
       CGGCGACGGG ACTATTGTAG GTGGTGGCGT CTTATTCGGT GTGGGTCGGT

33801  ACCTACACAT TCGTTCTGCG AGTCACACAC GGGAGGAGCG GGAAGAGCTG
       TGGATGTGTA AGCAAGACGC TCAGTGTGTG CCCTCCTCGC CCTTCTCGAC

33851  GAAGAACCAT GTTTTTTTTT TTATTCCAAA AGATTATCCA AAACCTCAAA
       CTTCTTGGTA CAAAAAAAAA AATAAGGTTT TCTAATAGGT TTTGGAGTTT

33901  ATGAAGATCT ATTAAGTGAA CGCGCTCCCC TCCGGTGGCG TGGTCAAACT
       TACTTCTAGA TAATTCACTT GCGCGAGGGG AGGCCACCGC ACCAGTTTGA
```

FIG.9A-40

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33951  CTACAGCCAA AGAACAGATA ATGGCATTTG TAAGATGTTG CACAATGGCT
       GATGTCGGTT TCTTGTCTAT TACCGTAAAC ATTCTACAAC GTGTTACCGA

34001  TCCAAAAGGC AAACGGCCCT CACGTCCAAG TGGACGTAAA GGCTAAACCC
       AGGTTTTCCG TTTGCCGGGA GTGCAGGTTC ACCTGCATTT CCGATTTGGG

34051  TTCAGGGTGA ATCTCCTCTA TAAACATTCC AGCACCTTCA ACCATGCCCA
       AAGTCCCACT TAGAGGAGAT ATTTGTAAGG TCGTGGAAGT TGGTACGGGT

34101  AATAATTCTC ATCTCGCCAC CTTCTCAATA TATCTCTAAG CAAATCCCGA
       TTATTAAGAG TAGAGCGGTG GAAGAGTTAT ATAGAGATTC GTTTAGGGCT

34151  ATATTAAGTC CGGCCATTGT AAAAATCTGC TCCAGAGCGC CCTCCACCTT
       TATAATTCAG GCCGGTAACA TTTTTAGACG AGGTCTCGCG GGAGGTGGAA

34201  CAGCCTCAAG CAGCGAATCA TGATTGCAAA AATTCAGGTT CCTCACAGAC
       GTCGGAGTTC GTCGCTTAGT ACTAACGTTT TTAAGTCCAA GGAGTGTCTG

34251  CTGTATAAGA TTCAAAAGCG GAACATTAAC AAAAATACCG CGATCCCGTA
       GACATATTCT AAGTTTTCGC CTTGTAATTG TTTTTATGGC GCTAGGGCAT

34301  GGTCCCTTCG CAGGGCCAGC TGAACATAAT CGTGCAGGTC TGCACGGACC
       CCAGGGAAGC GTCCCGGTCG ACTTGTATTA GCACGTCCAG ACGTGCCTGG

34351  AGCGCGGCCA CTTCCCCGCC AGGAACCATG ACAAAAGAAC CCACACTGAT
       TCGCGCCGGT GAAGGGGCGG TCCTTGGTAC TGTTTTCTTG GGTGTGACTA

34401  TATGACACGC ATACTCGGAG CTATGCTAAC CAGCGTAGCC CCGATGTAAG
       ATACTGTGCG TATGAGCCTC GATACGATTG GTCGCATCGG GGCTACATTC

34451  CTTGTTGCAT GGGCGGCGAT ATAAAATGCA AGGTGCTGCT CAAAAAATCA
       GAACAACGTA CCCGCCGCTA TATTTTACGT TCCACGACGA GTTTTTTAGT

34501  GGCAAAGCCT CGCGCAAAAA AGAAAGCACA TCGTAGTCAT GCTCATGCAG
       CCGTTTCGGA GCGCGTTTTT TCTTTCGTGT AGCATCAGTA CGAGTACGTC

34551  ATAAAGGCAG GTAAGCTCCG GAACCACCAC AGAAAAAGAC ACCATTTTTC
       TATTTCCGTC CATTCGAGGC CTTGGTGGTG TCTTTTTCTG TGGTAAAAAG

34601  TCTCAAACAT GTCTGCGGGT TTCTGCATAA ACACAAAATA AAATAACAAA
       AGAGTTTGTA CAGACGCCCA AAGACGTATT TGTGTTTTAT TTTATTGTTT

34651  AAAACATTTA AACATTAGAA GCCTGTCTTA CAACAGGAAA AACAACCCTT
       TTTTGTAAAT TTGTAATCTT CGGACAGAAT GTTGTCCTTT TTGTTGGGAA

34701  ATAAGCATAA GACGGACTAC GGCCATGCCG GCGTGACCGT AAAAAAACTG
       TATTCGTATT CTGCCTGATG CCGGTACGGC CGCACTGGCA TTTTTTTGAC

34751  GTCACCGTGA TTAAAAAGCA CCACCGACAG CTCCTCGGTC ATGTCCGGAG
       CAGTGGCACT AATTTTTCGT GGTGGCTGTC GAGGAGCCAG TACAGGCCTC
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FIG.9A-41

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34801  TCATAATGTA AGACTCGGTA AACACATCAG GTTGATTCAC ATCGGTCAGT
       AGTATTACAT TCTGAGCCAT TTGTGTAGTC CAACTAAGTG TAGCCAGTCA

34851  GCTAAAAAGC GACCGAAATA GCCCGGGGGA ATACATACCC GCAGGCGTAG
       CGATTTTTCG CTGGCTTTAT CGGGCCCCCT TATGTATGGG CGTCCGCATC

34901  AGACAACATT ACAGCCCCCA TAGGAGGTAT AACAAAATTA ATAGGAGAGA
       TCTGTTGTAA TGTCGGGGGT ATCCTCCATA TTGTTTTAAT TATCCTCTCT

34951  AAAACACATA AACACCTGAA AAACCCTCCT GCCTAGGCAA AATAGCACCC
       TTTTGTGTAT TTGTGGACTT TTTGGGAGGA CGGATCCGTT TTATCGTGGG

35001  TCCCGCTCCA GAACAACATA CAGCGCTTCC ACAGCGGCAG CCATAACAGT
       AGGGCGAGGT CTTGTTGTAT GTCGCGAAGG TGTCGCCGTC GGTATTGTCA

35051  CAGCCTTACC AGTAAAAAAG AAAACCTATT AAAAAAACAC CACTCGACAC
       GTCGGAATGG TCATTTTTTC TTTTGGATAA TTTTTTTGTG GTGAGCTGTG

35101  GGCACCAGCT CAATCAGTCA CAGTGTAAAA AAGGGCCAAG TGCAGAGCGA
       CCGTGGTCGA GTTAGTCAGT GTCACATTTT TTCCCGGTTC ACGTCTCGCT

35151  GTATATATAG GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA
       CATATATATC CTGATTTTTT ACTGCATTGC CAATTTCAGG TGTTTTTTGT

35201  CCCAGAAAAC CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC
       GGGTCTTTTG GCGTGCGCTT GGATGCGGGT CTTTGCTTTC GGTTTTTTGG

35251  CACAACTTCC TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTCACTTCC
       GTGTTGAAGG AGTTTAGCAG TGAAGGCAAA AGGGTGCAAT GCAGTGAAGG

35301  CATTTTAAGA AAACTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA
       GTAAAATTCT TTTGATGTTA AGGGTTGTGT ATGTTCAATG AGGCGGGATT

35351  AACCTACGTC ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC
       TTGGATGCAG TGGGCGGGGC AAGGGTGCGG GGCGCGGTGC AGTGTTTGAG

35401  CACCCCCTCA TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG
       GTGGGGGAGT AATAGTATAA CCGAAGTTAG GTTTTATTCC ATATAATAAC

                PacI
               ~~~~~~~~
35451  ATGATGTTAA TTAAGAATTC GGATCTGCGA CGCGAGGCTG GATGGCCTTC
       TACTACAATT AATTCTTAAG CCTAGACGCT GCGCTCCGAC CTACCGGAAG

35501  CCCATTATGA TTCTTCTCGC TTCCGGCGGC ATCGGGATGC CCGCGTTGCA
       GGGTAATACT AAGAAGAGCG AAGGCCGCCG TAGCCCTACG GGCGCAACGT

35551  GGCCATGCTG TCCAGGCAGG TAGATGACGA CCATCAGGGA CAGCTTCAAG
       CCGGTACGAC AGGTCCGTCC ATCTACTGCT GGTAGTCCCT GTCGAAGTTC
```

FIG.9A-42

35601 GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC
CGGTCGTTTT CCGGTCCTTG GCATTTTTCC GGCGCAACGA CCGCAAAAAG

35651 CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA
GTATCCGAGG CGGGGGGACT GCTCGTAGTG TTTTTAGCTG CGAGTTCAGT

35701 GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG
CTCCACCGCT TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC

35751 GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
CTTCGAGGGA GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG

35801 CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG
GACAGGCGGA AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC

35851 CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG
GACATCCATA GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC

35901 TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT
ACGTGCTTGG GGGGCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA

35951 CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC
GCAGAACTCA GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG

36001 CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT
GTGACCATTG TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA

36051 TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT
AGAACTTCAC CACCGGATTG ATGCCGATGT GATCTTCCTG TCATAAACCA

36101 ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC
TAGACGCGAG ACGACTTCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG

36151 TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA
AACTAGGCCG TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT

36201 AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC
TCGTCGTCTA ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT AGGAAACTAG

36251 TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT
AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA

36301 TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATC
AAACCAGTAC TCTAATAGTT TTTCCTAGAA GTGGATCTAG GAAAATTTAG

36351 AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA
TTAGATTTCA TATATACTCA TTTGAACCAG ACTGTCAATG GTTACGAATT

36401 TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT
AGTCACTCCG TGGATAGAGT CGCTAGACAG ATAAAGCAAG TAGGTATCAA

FIG.9A-43

```
36451  GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC
       CGGACTGAGG GGCAGCACAT CTATTGATGC TATGCCCTCC CGAATGGTAG

36501  TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG
       ACCGGGGTCA CGACGTTACT ATGGCGCTCT GGGTGCGAGT GGCCGAGGTC

36551  ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT
       TAAATAGTCG TTATTTGGTC GGTCGGCCTT CCCGGCTCGC GTCTTCACCA

36601  CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC
       GGACGTTGAA ATAGGCGGAG GTAGGTCAGA TAATTAACAA CGGCCCTTCG

36651  TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG
       ATCTCATTCA TCAAGCGGTC AATTATCAAA CGCGTTGCAA CAACGGTAAC

36701  CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC
       GATGTCCGTA GCACCACAGT GCGAGCAGCA AACCATACCG AAGTAAGTCG

36751  TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA
       AGGCCAAGGG TTGCTAGTTC CGCTCAATGT ACTAGGGGGT ACAACACGTT

36801  AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG
       TTTTCGCCAA TCGAGGAAGC CAGGAGGCTA GCAACAGTCT TCATTCAACC

36851  CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT
       GGCGTCACAA TAGTGAGTAC CAATACCGTC GTGACGTATT AAGAGAATGA

36901  GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA
       CAGTACGGTA GGCATTCTAC GAAAAGACAC TGACCACTCA TGAGTTGGTT

36951  GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT
       CAGTAAGACT CTTATCACAT ACGCCGCTGG CTCAACGAGA ACGGGCCGCA

37001  CAACACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC
       GTTGTGCCCT ATTATGGCGC GGTGTATCGT CTTGAAATTT TCACGAGTAG

37051  ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT
       TAACCTTTTG CAAGAAGCCC CGCTTTTGAG AGTTCCTAGA ATGGCGACAA

37101  GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT
       CTCTAGGTCA AGCTACATTG GGTGAGCACG TGGGTTGACT AGAAGTCGTA

37151  CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT
       GAAAATGAAA GTGGTCGCAA AGACCCACTC GTTTTTGTCC TTCCGTTTTA

37201  GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT
       CGGCGTTTTT TCCCTTATTC CCGCTGTGCC TTTACAACTT ATGAGTATGA

37251  CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA
       GAAGGAAAAA GTTATAATAA CTTCGTAAAT AGTCCCAATA ACAGAGTACT
```

FIG.9A-44

```
37301  GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG
       CGCCTATGTA TAAACTTACA TAAATCTTTT TATTTGTTTA TCCCCAAGGC

37351  CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT
       GCGTGTAAAG GGGCTTTTCA CGGTGGACTG CAGATTCTTT GGTAATAATA

37401  CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC
       GTACTGTAAT TGGATATTTT TATCCGCATA GTGCTCCGGG AAAGCAGAAG

37451  AAGAATTGGA TCCGAATTCT TAAT
       TTCTTAACCT AGGCTTAAGA ATTA
```

FIG.9A-45

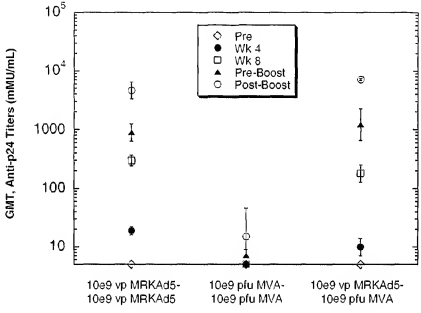
GMT, Anti-p24 Titers (mMU/mL)
10^5
10^4
1000
100
10
Pre
Wk 4
Wk 8
Pre-Boost
Post-Boost
10e9 vp MRKAd5-
10e9 vp MRKAd5
10e9 pfu MVA-
10e9 pfu MVA
10e9 vp MRKAd5-
10e9 pfu MVA

FIG. 10

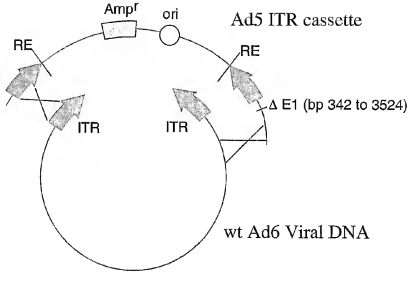
Amp^r
ori
Ad5 ITR cassette
RE
RE
Δ E1 (bp 342 to 3524)
ITR
ITR
wt Ad6 Viral DNA

FIG. 11

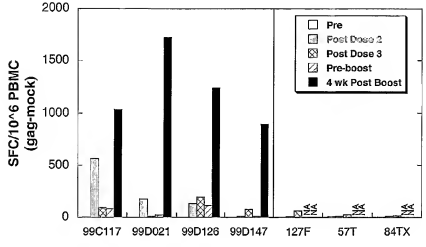
2000
1500
1000
500
0
SFC/10^6 PBMC (gag-mock)
Pre
Post Dose 2
Post Dose 3
Pre-boost
4 wk Post Boost
99C117
99D021
99D126
99D147
127F
57T
84TX

FIG. 12